Chemokine modulation of hippocampal function across aging

Annabel Florence Smith

PhD

University of York

Biology

February 2022

Abstract

The hippocampus undergoes several structural, cellular, and functional changes during normal aging which is directly associated with cognitive decline and the pathogenesis of neurodegenerative disease. Dysregulation of innate immune function in the peripheral and central nervous system during aging is thought to contribute to reduced cognition and the pathophysiological events leading to neurodegeneration. Most studies of aging compare young individuals with elderly, however there is limited investigation into whether hippocampal function also declines in early aging.

In this thesis I used *in vitro* electrophysiology on mouse hippocampal slices to examine hippocampal excitability along the perforant path in an early aging model, comparing neuronal activity between juvenile (9-15 weeks old) and adult (25-35 weeks old) C57BL/6J mice. I also examined if the Duffy antigen receptor for chemokines (DARC), which mediates circulating pro-inflammatory chemokine concentration, was affecting hippocampal excitability using homozygous DARC knockout mice.

Extracellular measurements of dentate gyrus neurons during perforant pathway stimulation showed that hippocampal excitability increases in early aging. Intracellular measurements of intrinsic membrane properties indicated that dentate gyrus granule neurons become hyperpolarised and have increased membrane resistance during early aging, which occurred only in the supra-pyramidal subregion. Bath application of chemokine CCL2 increased hippocampal excitability via its cognate receptor CCR2, however not in an age-dependent manner, indicating that CCL2 does not regulate early age-dependent excitability. Measurements of population excitability and granule cell intrinsic properties in DARC knockout mice indicated that DARC regulates neuronal excitability in early aging and resting membrane potential of supra-pyramidal granule neurons. Adult DARC knockout mice also had increased hippocampal microglia proliferation. These are the first reported effects of DARC deficiency on brain cells.

These implications of these findings, for our overall understanding of how hippocampal function changes in early aging and how chemokines and chemokine receptors alter hippocampal excitability, are discussed.

Table of Contents

List of figures

Acknowledgments

I can't quite believe it's finished; I am relieved but also sad that this journey is over. It's been a long and eventful ride, and there are many people that I would like to thank.

I would firstly like to acknowledge and thank my industrial partner Eisai Ltd.

A very big and grateful thank you to all my supervisors. Firstly, Sangeeta. Thank you for your support, enthusiasm, and for taking me under your wing. I did very much enjoy my time becoming a member of the D1 corridor. Your very fun dinner parties and rather tasty apple chutney certainly deserve a shoutout. Thank you to Darren for your help, guidance, and support throughout my 4 years of study. Nathalie, thank you for your invaluable immunity knowledge, technical and emotional support. A very big thank you to my TAP and progression members Will and Sean for your help and engagement throughout my project.

To Miles, not only a supervisor but also a mentor. Your passion and enthusiasm for science was infectious, along with your humour and ways of working. I will always be grateful for your fierce support and encouragement, and for building a thoroughly enjoyable and family-like lab environment. It was a pleasure to have the opportunity to work side by side with you on the rigs. You will be missed.

To the Long B0i's gals. First of all, Grace (the OG). We've been on this journey together since day one, and I can't imagine how I would have muddled my way through without you. Here's to all those hours in the lab huddled around that storage heater and sharing our love for Ru Paul. To Grace (Keanu Reeves), the best flatmate a girl could ask for. I'll miss our wine evenings, laptops on our laps, binge-watching RHOBH. I don't like to admit this, but I ended up quite liking the plants in the end. Jess and Sarah, you are rays of sunshine, beautiful chaotic rays of sunshine. I wouldn't have wanted to experience this journey without any of you.

A big thank you to the previous Whittington Lab members. Steve, Anna, Iain and the honorary member Nick. I thoroughly enjoyed our trips to the Charles, Big Eat, Vanbrugh Burritos, Fish and Chip Fridays…why were we were all so obsessed with food? To Steve, not only did you teach me all I needed to know about ephys, but you're also now teaching

me everything I need to know about market access consultancy. Who would have thought?

To my family and friends, thank you for your undeniable support and encouragement throughout this process. To Roo, thank you for your hilarious phone calls and always making me laugh. To Mum, thank you for your upmost patience and always lending an ear when I needed it the most. Thank you for your many thoughtful gestures, your trips to York to take me out for lunch when I was worn out, and for being amazing as always.

To Taylor, my knight in shining armour. Thank you for looking after me during my write up period, cooking every meal and making every coffee. In fact, thank you for looking after me throughout the whole 4 year process. I promise we can leave the flat now and have some fun. I love you.

And to Dad, I know you would be proud of me because you used to tell me every day. This one's for you.

Author’s Declaration

I declare that this thesis is a presentation of original work, and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References

Chapter 1 - General Introduction

1.1 The hippocampus

1.1.1 Overview

The hippocampal formation is one of the most studied neuronal systems in the brain as it plays an important role in episodic memory, spatial navigation, anxiety related-behaviour, and is involved in regulation of hypothalamic functions (Stella *et al.*, 2012; Toyoda *et al.*, 2011; Koehl and Abrous, 2011). The hippocampus is vulnerable to age-related changes in circuitry (Barnes, Rao and Houston, 2000a; Burke and Barnes, 2010; Yassa, Muftuler and Stark, 2010), volume (Driscoll *et al.*, 2006), neuronal number (Fu *et al.*, 2015), and associated behaviour (Kausler, 1994; Gage *et al.*, 1984; Rapp, Kansky and Roberts, 1997; Newman and Kaszniak, 2000). Moreover, the hippocampus is affected in neurodegenerative diseases such as Alzheimer's disease and Parkinson's related dementia (Ritchie *et al.*, 2018; Camicioli *et al.*, 2003). Evidence has emerged that changes in hippocampal function and related behaviours are altered in midlife (Williams *et al.*, 2019; Hattiangady *et al.*, 2005), suggesting that middle age could be a critical period in the development of pathophysiological mechanisms leading to neurodegenerative diseases later in life (Ritchie *et al.*, 2018). Despite this, study into whether aging-pathologies occur progressively with aging is scarce. Further investigation into hippocampal function with early aging could help to identify physiological functional changes that pre-depose individuals to age-related disease and provide a potential opportunity for preventative intervention.

1.1.2 Hippocampal memory function

1.1.2.1 Episodic memory

There is general agreement amongst the scientific community that the hippocampus is essential for episodic memory - the memory of everyday events that are the collection of past personal experiences (Wiggs, Weisberg and Martin, 1999; Eichenbaum, 2000) and was first described by Endel Tulving in 1972 (Tulving, 1984; Tulving, 1985; Clayton, Salwiczek and Dickinson, 2007). Episodic memory requires binding together of stimuli, along with spatial, temporal and conceptual relationships to form coherent memory representations (Pender, 2002). It is often contrasted with semantic memory, which includes memory for generic, context free knowledge (Hudson, Mayhew and Prabhakar, 2011). Nine properties of episodic memory have been described to distinguish it from other forms of memory: it has the perspective of the observer; represents short time-

frames of experience; contains summary records of sensory-perceptual-conceptual-affective processing; often represented in the form of visual images; retains patterns of activation/inhibition over long periods; represented on a temporal dimension in an approximate order of occurrence; is subject to rapid forgetting; makes autographical remembering specific and is collectively experienced when accessed (Conway, 2009).

1.1.2.2 Spatial navigation

The hippocampus also plays a role in spatial navigation and memory, which was first identified through the observation that hippocampal neurons exhibit spatially localised firing patterns when rats explore their environments (O'Keefe and Dostrovsky, 1971). It was later observed that damage to the hippocampus and amygdala in primates caused a severe impairment in object recognition (Mishkin, 1978), highlighting that the hippocampus also plays a role in visual memory. Strong activation of the hippocampus has been routinely observed during memory guided spatial navigation experiments (Maguire, Frackowiak and Frith, 1997; Bontempi *et al.*, 1999), and activity in hippocampal pyramidal neurons has been shown to be location specific (O'Keefe and Dostrovsky, 1971; Wilson and McNaughton, 1993).

Pyramidal neurons in the hippocampus and granule cells in the dentate gyrus act as place cells that become active when an animal enters a space in an environment, known as a place field. Place cells are a cognitive representation of a specific location in space (Routtenberg, 1980), and communicate with other neurons in the hippocampus and surrounding regions to perform spatial processing activities (Muir and Bilkey, 2001). In addition, grid cells in the entorhinal cortex fire at regular intervals as an individual navigates an open area, allowing the storage of information about location, distance, and direction (Fyhn *et al.*, 2004; Hafting *et al.*, 2005). The idea that the hippocampus is involved in generating a mental representation of the spatial layout of an environment is supported by a number of reports indicating that hippocampus neurons are acutely sensitive to the spatial geometry of an environment (Gothard *et al.*, 1996; Muller and Kubie, 1987). In particular, hippocampal-dependent spatial navigation is thought to depend on the arrangement of distal landmarks (Muller, Kubie and Ranck, 1987; O'Keefe and Speakman, 1987; O'Keefe and Burgess, 1996), with navigation still partially successful after removal or concealment of the controlling cues (Quirk, Muller and Kubie, 1990; O'Keefe and Speakman, 1987). Hippocampal response patterns are also sensitive

to non-geometric features, with neurons exhibiting changes in spatial firing patterns in response to an environment's odour and colour, highlighting that spatial geometry cannot solely be determined by hippocampal responses (Anderson and Jeffery, 2003; Hayman *et al.*, 2003).

Animals with hippocampal lesions fail to learn the location of a hidden goal whose position is defined by the arrangement of distal landmarks (Jarrard, 1978; Olton, Walker and Gage, 1978; Morris *et al.*, 1982; Sutherland, Whishaw and Kolb, 1983). The size of the hippocampal lesion affects spatial navigation and object recognition differently; lesions in the dorsal hippocampus damaging 30-50% of total hippocampal volume cause severe spatial memory impairment, yet object recognition memory is only impaired to the same extent after a lesion volume of 75-100% total volume. Recognition memory was entirely spared by smaller lesions (Moser *et al.*, 1995; Broadbent, Squire and Clark, 2004). However not all features of spatial navigation and memory depend on the hippocampus; rats with hippocampal lesions can solve landmark-based navigation tasks under certain conditions, such as when training is prolonged or when the task difficulty is progressively increased (Morris *et al.*, 1990; Whishaw, Cassel and Jarrad, 1995). Evidence suggests that the hippocampus is essential for goal-directed spatial navigation as hippocampal lesions cause a severe but selective deficit in the identification of a location, suggesting that the hippocampus is essential for image recognition during spatial navigation (Hollup *et al.*, 2001). (Hollup *et al.*, 2001)

1.1.2.3 Emotional and social processing

The hippocampus is involved in emotional processing and is reported to be distinct from that of the amygdala, which is suggested to be associated with fear rather than anxiety (Bannerman *et al.*, 2004). Early support for this view was discovered when the removal of the medial temporal lobe caused profound emotional disturbance in monkeys (Klüver and Bucy, 1937). The region exerts strong regulatory control of the hypothalamic-pituitary-adrenal (HPA) axis, a complex set of direct influences and feedback mechanism interactions between the hypothalamus, pituitary gland and adrenal glands. It constitutes a major neuroendocrine system controlling reactions to stress and regulates body processes such as the immune system, emotions, energy storage and expenditure (Jacobson and Sapolsky, 1991). Glucocorticoid hormones are secreted from the adrenal gland and stress-induced rise in glucocorticoid concentration facilitates adaptation to

stress and restores homeostasis through the enhancement and promotion of motivational and cognitive processes (de Kloet, Joëls and Holsboer, 2005; Joëls *et al.*, 2007; McEwen, 2007). These hormones can enter the brain and bind to two intracellular receptor types, mineralocorticoid receptors and glucocorticoid receptors that regulate transcription of response genes. These are both found in abundance in the hippocampus (Joëls, 2001; Meijer, Buurstede and Schaaf, 2019). Excessive, prolonged or inadequate glucocorticoid response impairs an individual's adaptation to stress and is considered a risk factor for disease, having a profound effect on brain development (de Kloet, Karst and Joëls, 2008). Hippocampal lesions have shown to severely impair control of the hormonal stress response (Dedovic *et al.*, 2009), and elevations of stress hormones lead to hippocampal dysfunction in rodents and humans (McEwen *et al.*, 1997; Herman *et al.*, 2005). Decreased hippocampal volume and general hippocampal dysfunction are associated with psychological disorders such as post-traumatic stress disorder, bipolar disorder and depression, with effective pharmacological treatments of these disorders targeting hippocampal function and physiology (Bonne *et al.*, 2008; Frey *et al.*, 2007). The hippocampus is also involved in the formation of emotional memories in social contexts (Eisenberger, Gable and Lieberman, 2007) and in the processing of emotional facial expressions (Critchley *et al.*, 2000; Fusar-Poli *et al.*, 2009).

Social memory refers to the ability to retrieve socially relevant information from past experiences and encounters. Social recognition teaches exploration of unfamiliar individuals and encourages adaptation to familiar individuals, promotes reproductive behaviours and enables recognition of social hierarchical positions (Montagrin, Saiote and Schiller, 2018). The hippocampus is essential for learning new information about a person that is tied to a specific event or experience, contributes to the ability to form relationships with others, influence behaviour in social situations and effects judgements and perceptions (Rubin *et al.*, 2014). Animal studies show a link between the hippocampus and social recognition. In rodents, social recognition is important for mate choice and maintaining social hierarchy (Berry and Bronson, 1992), and hippocampal lesions impair the robust recognition that adult mice exhibit toward young conspecifics and affect the distinct grooming behaviour degus display towards familiar and unfamiliar partners (Kogan, Frankland and Silva, 2000; Uekita and Okanoya, 2011). In addition, social isolation in mice and rats induces impairment of hippocampal-dependent long term

social recognition memory (Kogan, Frankland and Silva, 2000; Pena *et al.*, 2014). In non-human primates, a single-unit study showed that hippocampal-neurons expressed a significant activity enhancement to faces and voices (Sliwa *et al.*, 2016) and single-unit recordings of human hippocampal cells show they fire in a highly selective and invariant manner for familiar and well-known individuals (Quiroga *et al.*, 2005; Quiroga *et al.*, 2009; Viskontas, Quiroga and Fried, 2009). Functional magnetic resonance imaging of primates shows the hippocampus as an area with enhanced activity for faces compared with objects (Ku *et al.*, 2011; Lafer-Sousa and Conway, 2013), and functional neuroimaging of healthy humans undertaking a facial recognition task showed that hippocampal activation correlated with how well participants recognised the faces presented (Trinkler *et al.*, 2009). The study of human patients with hippocampal impairments show difficulty retrieving sematic information about well-known individuals, possess difficulty in establishing interpersonal relationships and present limited social circles compared with unimpaired controls (Sanders and Warrington, 1971; Davidson *et al.*, 2012). Restricted social circles could be explained by the inability to remember faces and difficulties in accessing episodic memories, such as the details of a moment shared with a person (McHugh *et al.*, 2004).

1.1.3 Hippocampal Structure and circuitry

The hippocampus lies deep in the medial and temporal lobes and can be distinguished externally as a layer of densely packed neurons which curl into a small S-shaped structure, measuring approximately 3-3.5cm^3 in volume on each side of the brain (Anand and Dhikav, 2012; Gilbert and Brushfield, 2009; Bird and Burgess, 2008). The structure of the hippocampus is conserved across mammals and runs along a dorsal (septal) to ventral (temporal) axis in rodents, which corresponds to a posterior to anterior axis in humans (Figure 1.1). The same basic intrinsic circuitry is maintained throughout the long axis across species; however, the 3D nature of the hippocampus gives rise to the dorsal and ventral portions of the hippocampus which have different connections with cortical and subcortical areas (Strange *et al.*, 2014). The dorsal hippocampus receives polymodal sensory information from cortical areas; the ventral hippocampus is more closely linked to sub-cortical structures such as the amygdala and hypothalamic-pituitary axis (Bannerman *et al.*, 2014; Naber and Witter, 1998).

Memory formation and recall requires interactions between the entorhinal cortex and dentate gyrus, forming a cortico-hippocampal circuit. This circuit receives multisensory inputs from the olfactory domain of the telencephalon, perirhinal cortex, and pre- and para-subiculum to the superficial layers of the entorhinal cortex, which then routes inputs into the hippocampus. The hippocampus can be subdivided into the Cornu ammonis regions (CA), dentate gyrus (DG), subiculum and entorhinal cortex. The Cornu ammonis is further subdivided into the CA1, CA2 and CA3 in rodents, with the addition of the CA4 in the human brain (Figure 1.1 Aii and Bii) (Anand and Dhikav, 2012).

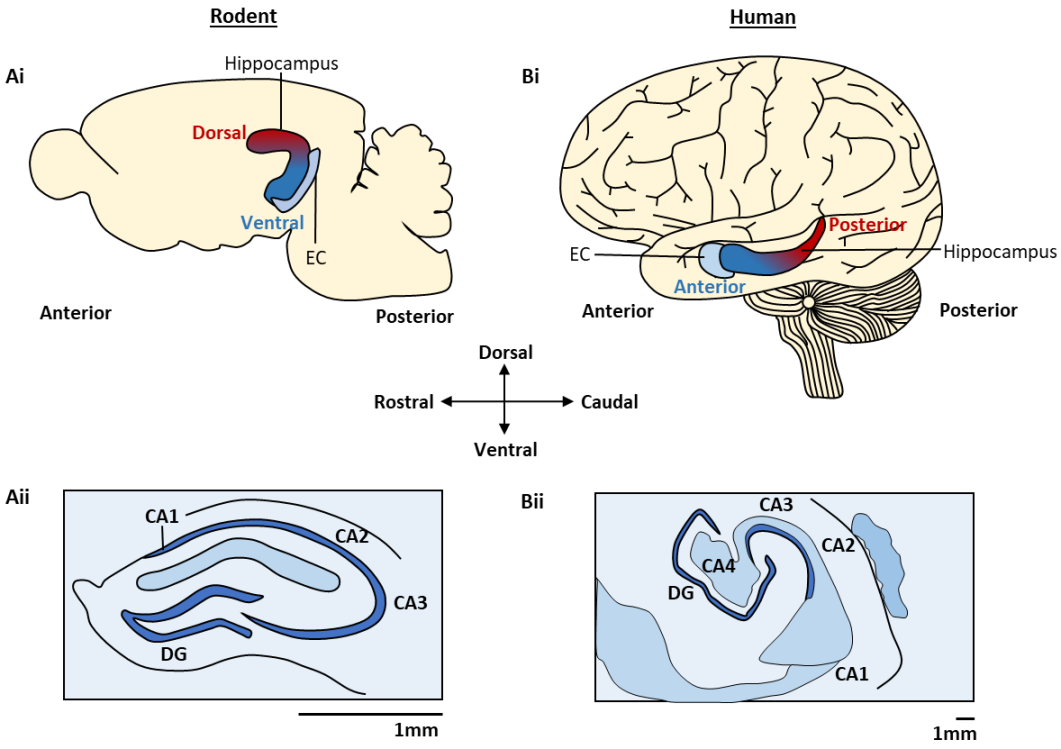


Figure 1.1 Rodent and human cross-species comparison of hippocampal anatomy. The location and shape of the longitudinal axis of the hippocampus is shown in the rodent brain (Ai) and human brain (Bi). The longitudinal axis is described as ventro-dorsal in rodents and antero-posterior in humans. A 90 degree rotation is required for the rodent hippocampus to have the same orientation as that of humans. Figures Aii and Bii show schematic diagrams of Nissl stained cross-sections of a rodent and human hippocampi, respectively. Figure adapted from Strange *et al.*, 2014 and O'Leary and Cryan, 2014.

1.1.3.1 The perforant pathway

The entorhinal cortex is the major input and output structure of the hippocampus, forming the nodal point in cortico-hippocampal circuits (Witter *et al.*, 1989). Sensory inputs converge at the neuronal level in the superficial layers of the entorhinal cortex layer II and layer III which then project to the hippocampus (van Strien, Cappaert and Witter, 2009; Naber *et al.*, 1997). Intrinsic inputs from the entorhinal cortex layer III project directly to the CA1, whilst inputs arising in the entorhinal cortex layer II instead project to the dentate gyrus, known as the perforant pathway (Buzsaki and Moser, 2013; Canto, Wouterlood and Witter, 2008; Hyman *et al.*, 1986). The dentate gyrus then routes these inputs to the CA3 via the mossy fibres, a projection formed of granule cell axons (Steward, 1976; Blackstad *et al.*, 1970), and then through Schaffer collateral synapses to the CA1 (Witter, 2007). The CA1 and subiculum send back processed memory output to the deep layer V of the entorhinal cortex as well as less dense projection to layers II and III, which then communicate with other brain structures (van Strien, Cappaert and Witter, 2009; Basu and Siegelbaum, 2015; de No, 1933). This circuitry is described as the hippocampal tri-synaptic loop and is summarised in figure 1.2.

Principal neurons in the dentate gyrus receive mainly monosynaptic inputs from the entorhinal cortex, whereas pyramidal cells in the CA3 and CA1 receive monosynaptic inputs via perforant pathway fibres as well as additional input via the hippocampal tri-synaptic circuit. There is no report of backpropagation of signals form the CA3 to DG (Amaral, Scharfman and Lavenex, 2007).

1.1.3.2 The lateral and medial perforant pathway

The entorhinal cortex can be sub-divided into the medial entorhinal cortex (MEC) and the lateral entorhinal cortex (LEC) (Insausti, Herrero and Witter, 1997). The LEC is primarily innervated by the perirhinal cortex, whilst the MEC is primarily innervated by the postrhinal cortex, but also receives minor projections from the visual association areas and posterior parietal and retrosplenial areas (Burwell and Amaral, 1998; Burwell, 2000). The MEC and LEC generate the medial and lateral perforant pathways, respectively. The MEC terminates in the middle one third of the dentate gyrus molecular layer, whereas the LEC terminates on the outer third of the dentate gyrus molecular layer (McNaughton, 1980). The MEC and LEC are differentially involved in spatial learning and episodic memory (Ferbinteanu, Holsinger and McDonald, 1999).

Electrophysiological recordings demonstrate that cells in the MEC are predominantly spatially modulated, processing contextual and spatial environment information, involved in the brains *where* processing stream (Hargreaves *et al.*, 2005). In contrast, this modulation is absent in the LEC, and instead neuron firing has been correlated to objects in context and is part of the *what* processing stream (Fyhn *et al.*, 2004; Deshmukh and Knierim, 2011; Knierim, Neunuebel and Deshmukh, 2014; Moser *et al.*, 2014; Tsao, Moser and Moser, 2013). Episodic memory requires an integration of *what*, *where* and *when* components of a memory (Clayton and Dickinson, 1998; Eichenbaum and Fortin, 2005), highlighting that both LEC and MEC components are essential for the formation of episodic memory.

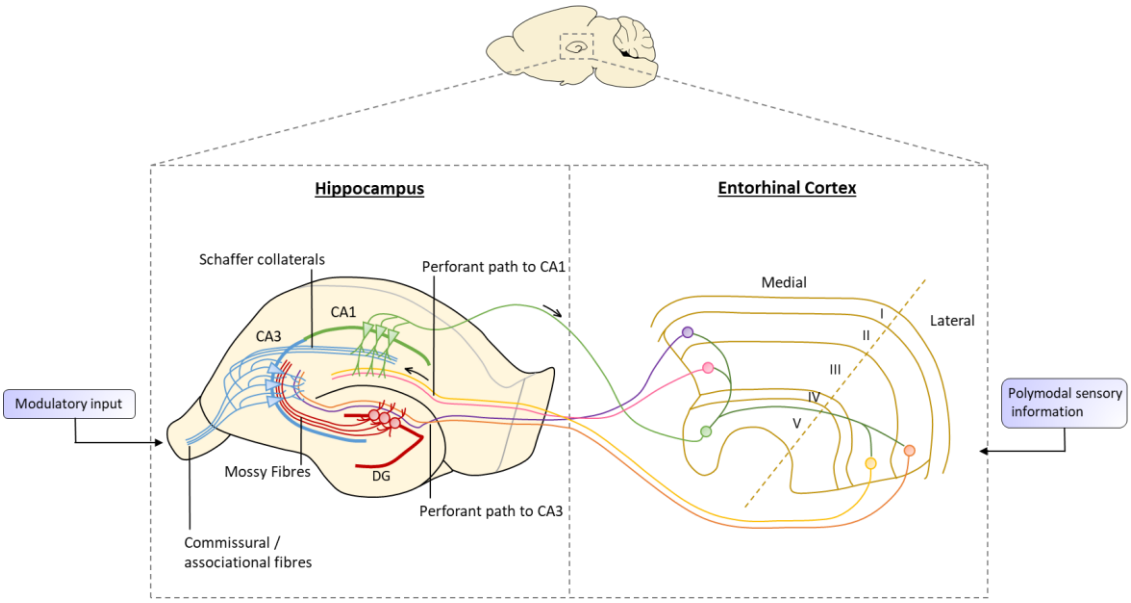


Figure 1.2 Basic circuitry of the hippocampus. Hippocampal basic circuitry is traditionally presented as a tri-synaptic loop, where major polymodal sensory information input is carried from neurones of the entorhinal cortex layer II to the dentate gyrus by axons of the perforant path. Perforant path axons make excitatory synaptic contact with the dendrites of dentate granule cells - axons from the lateral entorhinal cortex and medial entorhinal cortex innervate the outer third and middle third of the dendritic tree, respectively. Granule cells project through their axons, the mossy fibres, to the proximal apical dendrites of CA3 pyramidal cells. These project to ipsilateral CA1 pyramidal cells through Schaffer collaterals and to contralateral CA3 and CA1 pyramidal cells through commissural connections. The distal apical dendrites of CA1 pyramidal neurones also receive a direct input from EC layer III cells. The CA1 sends processed memory output to EC layer V and a less dense projection to layers II and III. Figure adapted from (Neves, Cooke and Bliss, 2008).

1.1.3.3 Dentate gyrus structure

The dentate gyrus is a laminar structure that comprises a dense layer of granule cells that possess a hyperpolarised resting membrane potential and low average firing rate (Nusser and Mody, 2002). The dentate gyrus acts to reduce interference between the entorhinal cortex and CA3, performing pattern separation by combing spatial and object information from the entorhinal cortex into a single representation of decorrelating input to the CA3 (Leutgeb *et al.*, 2007; O'Reilly and McClelland, 1994). The dentate gyrus is comprised of two subregions called the supra-pyramidal dentate gyrus, which lies closest to the CA1, and the infra-pyramidal dentate gyrus, as represented in figure 1.3 (Scharfman *et al.*, 2002). The supra-pyramidal and infra-pyramidal regions have been previously described as homogeneous, and it was originally reported that entorhinal cortex projections innervated both subregions equally (Tamamaki and Nojyo, 1993), with modulatory inputs into the dentate gyrus seemingly had no selectivity for either subregion (Frotscher, Zhao and Forster, 2007). More recent evidence suggest that the dentate gyrus may not be as uniform as previously defined.

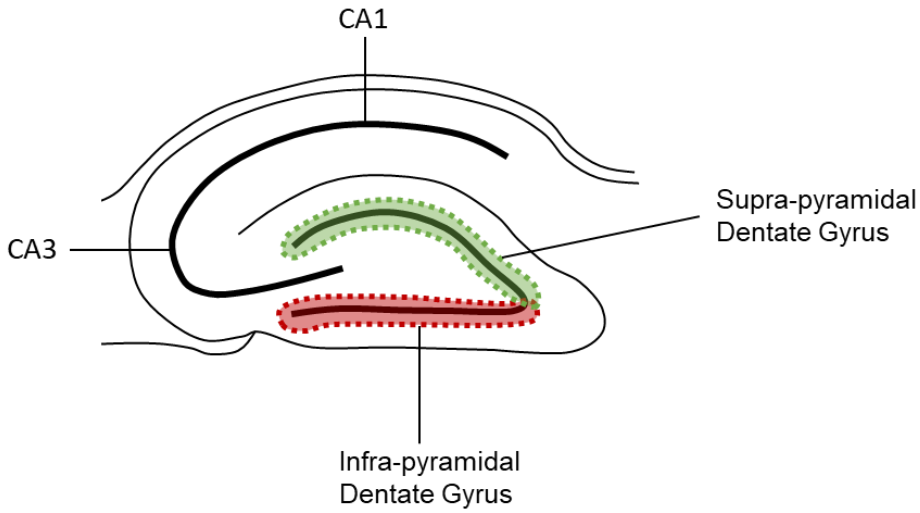


Figure 1.3 Dentate gyrus subregions. The dentate gyrus can be subdivided into the supra-pyramidal dentate gyrus (green) and the infra-pyramidal dentate gyrus (red). The subregions have been reported to have different circuit functions, with the supra-pyramidal dentate gyrus reported to be more active during spatial navigation. Figure adapted from (Greene *et al.*, 2013; Tamamaki, 1997).

Research suggests that the supra-pyramidal and infra-pyramidal subregions could contain different circuit functions (Scharfman *et al.*, 2002), as the supra-pyramidal blade is more active during spatial navigation, with the neuronal activity markers activity-dependent cytoskeleton associated protein (Arc) and early growth response protein 1 (ERG-1) showing 4-fold higher expression in the supra-pyramidal region compared to the infra-pyramidal region during rodent behavioural tasks (Chawla *et al.*, 2005; Satvat *et al.*, 2011). In general, the supra-pyramidal subregion is considered more active, and greater levels of c-fos mRNA, a marker of neuronal activity, are observed in the supra-pyramidal dentate gyrus compared to the infra-pyramidal (Chawla *et al.*, 2013).

The dentate gyrus subregions also have different cellular morphologies. Supra-pyramidal granule cells have greater dendritic length and spine densities (Desmond and Levy, 1982; Desmond and Levy, 1985; Claiborne, Amaral and Cowan, 1990), which are associated with are larger number of synaptic contacts (Sorra and Harris, 2000). A larger quantity of GABA-immunoreactive neurons are located in the supra-pyramidal dentate gyrus (Woodson, Nitecka and Benari, 1989; Czeh *et al.*, 2013), and these inhibitory neurons are more densely innervated by mossy fibres than infra-pyramidal dentate gyrus neurons (Ribak and Peterson, 1991). Furthermore, the infra-pyramidal dentate gyrus exhibits higher levels of cell proliferation than the suprapyramidal blade (Snyder, Ferrante and Cameron, 2012); however, the infra-pyramidal subregion also shows greater vulnerability to hypoxia (Hara *et al.*, 1990), cannabinoid treatment (Simonyi, Miller and Sun, 2000) and sleep deprivation, with larger reductions in spine density observed (Raven *et al.*, 2019). Although the supra-pyramidal subregion is more active, the infra-pyramidal subregion shows greater subregional excitability and less paired-pulse inhibition in rats with chronic seizures (Scharfman *et al.*, 2002), suggesting that the infra-pyramidal subregion is more susceptible to pathologic conditions that the supra-pyramidal region.

Single unit studies typically record in the supra-pyramidal subregion (Jung and McNaughton, 1993; Leutgeb *et al.*, 2007; Schmidt, Marrone and Markus, 2012). However, because multiple differences in excitability and vulnerability have been observed between the supra-pyramidal and infra-pyramidal dentate gyrus, it highlights that in order to achieve an accurate picture of dentate gyrus function, measurements in both subregions should be conducted.

1.1.4 Ventral and dorsal hippocampal function

In an influential review, it was suggested that the hippocampus may not act as a unitary structure, but instead have different roles associated with the dorsal and ventral hippocampal portions (Moser and Moser, 1998). Their reasoning was based on three major experimental findings. Firstly, anatomical studies indicated that the input and output connections of the dorsal and ventral hippocampus are distinct (Swanson and Cowan, 1977), secondly, spatial memory is dorsal- and not ventral-dependent (Moser *et al.*, 1995), and finally, ventral lesions alter stress responses and emotional behaviour and not dorsal regions (Henke, 1990). Although the basic circuitry of the hippocampus is similar along its dorsoventral axis, the main extrinsic and intrinsic connections are very different for dorsal and ventral regions, and their connections with different extra-hippocampal structures are summarised in figure 1.4. These connections are complex and involve the cross talk between multiple brain regions.

In addition to circuitry connections, neurochemical localisation is different between the dorsal and ventral hippocampus (Sousa, Cerqueira and Almeida, 2008), with cholinergic innervation being more present in the dorsal hippocampus (Amaral and Kurz, 1985; Milner, Loy and Amaral, 1983) and fibres containing dopamine, noradrenaline and serotonin being more present in the ventral hippocampus (Verney *et al.*, 1985; Gage and Thompson, 1980; Haring and Davis, 1985; Köhler, Chan-Palay and Steinbusch, 1981).

1.1.4.1 Dorsal hippocampal connectivity

The dorsal hippocampus receives and sends prominent cortical projections from the retrosplenial and anterior cingulated cortices, which are the two cortical regions primarily involved in the cognitive processing of visual-spatial information (Van Groen and Wyss, 2003; Vogt and Miller, 1983; Kobayashi and Amaral, 2007; Roberts *et al.*, 2007; Harker and Whishaw, 2004). Therefore, the dorsal hippocampus has been reported to be responsible for cognitive processing of visuospatial information and spatial navigation, which was identified by lesions in this region reducing visuospatial memory and navigation performance in rats (Harker and Whishaw, 2004), monkeys (Lavenex, Lavenex and Amaral, 2007; Kishi *et al.*, 2000) and humans (Maguire, Nannery and Spiers, 2006). Furthermore, parallel projections from the dorsal subiculum, which is positioned between the hippocampus and entorhinal cortex (O'Mara *et al.*, 2001), reach the lateral and medial mammillary nucleus and the anterior thalamic complex (Ishizuka,

2001), which contain a high number of navigation-related neurons (Taube, 2007). These subcortical structures send their projections back to the dorsal hippocampus and retrosplenial cortex (Risold, Thompson and Swanson, 1997), and this neural network provides the import circuitry needed to register a cognitive map for the navigation system, enabling animals to properly orient and execute behaviours in a learned environment (Muller, Stead and Pach, 1996; Taube, Muller and Ranck, 1990).

Functional magnetic resonance imaging (fMRI) studies of human participants highlight that encoding of visual information results in the greatest activation in the dorsal hippocampus, whilst information retrieval showed greater activation in the ventral hippocampus (Stern *et al.*, 1996; Gabrieli *et al.*, 1997). Staining for the expression of the c-Fos gene is an indirect correlate of increased neuronal activity and is induced during learning experiences (Sagar, Sharp and Curran, 1988; Tischmeyer and Grimm, 1999). cFos activation was discovered to be greater in the dorsal hippocampus dentate gyrus, CA3, CA1 and dorsal subiculum subregions when compared to the ventral hippocampus when mice underwent spatial working memory tasks using novel landmarks (Vann *et al.*, 2000).

1.1.4.2 Ventral hippocampal connectivity

The ventral hippocampus has different efferent connections than the dorsal hippocampus which gives rise to different associated behaviours. The ventral hippocampus is anatomically connected to structures that regulate neuroendocrine and behavioural responses to stress, anxiety, reward processing, motivation and executive function and social behaviour (Bannerman *et al.*, 2003; Bannerman *et al.*, 2004; McHugh *et al.*, 2004; Swanson and Cowan, 1977).

Bi-directional connectivity exists between the ventral hippocampus and the amygdala, an area of the brain associated with processing emotion, including fearful and threatening stimuli (Calder, Lawrence and Young, 2001). Efferent connectivity to the nucleus accumbens and medial prefrontal cortex modulates reward and emotional behaviour circuitry (Sahay and Hen, 2007; Liu and Carter, 2018; Liang *et al.*, 2018), and these structures along with the ventral hippocampus and amygdala form a series of parallel and segregated projections, either directly or indirectly through the medial and central amygdala nuclei, and bed nuclei of the stria terminalis (BST), to innervate the

hypothalamus (Fanselow and Dong, 2010). The hypothalamus is the primary structure involved in the hypothalamic-pituitary-adrenal axis, which controls neuroendocrine, autonomic, and somatic motor activities associated with three basic classes of motivated behaviours having strong emotional components including ingestion, reproduction, and defence (Dong, Petrovich and Swanson, 2001; Herman *et al.*, 2005; Kishi *et al.*, 2000).

Hippocampal lesions impair control of the hormonal stress response (Buchanan, Tranel and Kirschbaum, 2009) and in agreement, amygdala lesions and inactivation blocks stress effects on hippocampal long term potentiation and spatial memory in rats (Kim *et al.*, 2001; Kim *et al.*, 2005), and elevations of stress hormones, such as corticosterone, lead to hippocampal dysfunction in rodents (McEwen *et al.*, 1997). Furthermore, in humans, decreased hippocampal volumes and hippocampal dysfunction are associated with psychological disorders with strong affective components such as posttraumatic stress disorder, bipolar disorder, and depression (Frey *et al.*, 2007; Bonne *et al.*, 2008). Indeed, effective pharmacological treatments of these disorders target hippocampal function and physiology (Fanselow and Dong, 2010). These data highlight the extensive correlation with the hippocampus and emotion, an affect that is similarly striking as its relationship with memory.

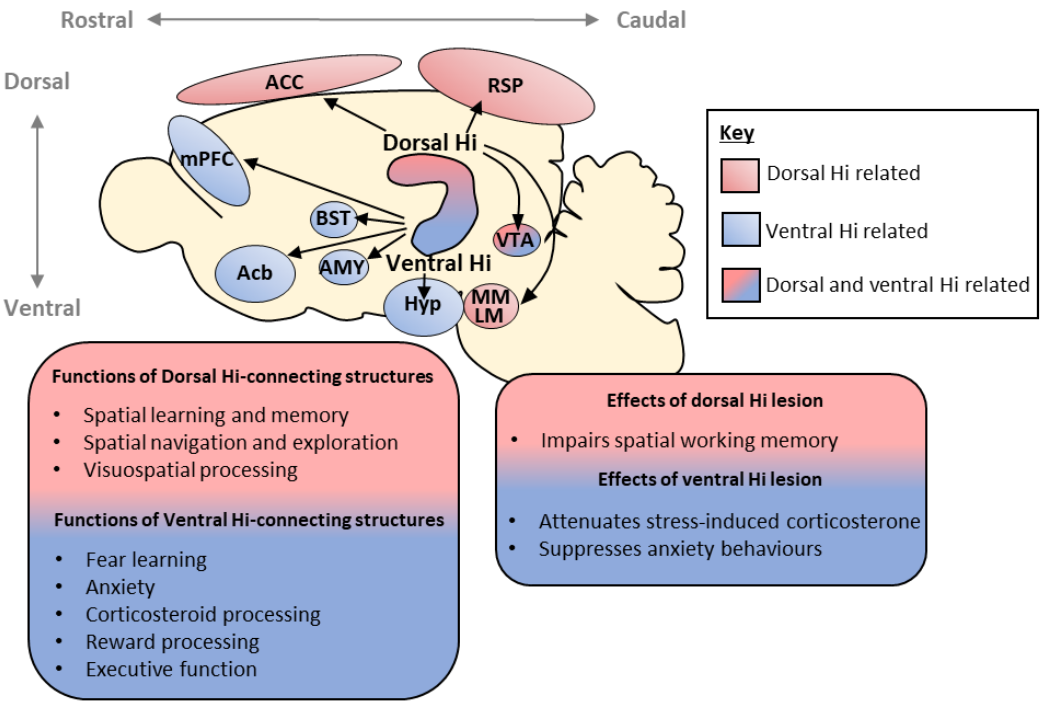


Figure 1.4 Segregation of the rodent hippocampus along the dorsoventral axis, its connectivity to other brain regions and subsequent functions. The rodent hippocampus (Hi) extends along a rostro-caudal and dorso-ventral axis, beginning at the septal nuclei forming a dorsal pole (pink) and ending in the temporal lobe to form a ventral pole (blue). The rodent hippocampus has distinct anatomical connections along the dorsoventral axis, as shown by arrows. Connecting structures of the dorsal hippocampus are displayed in pink and have roles in spatial learning and memory, navigation and visuospatial processing. The connecting structures of the ventral hippocampus are represented in blue and are regulators of stress hormone release, reward processing, motivation, anxiety and executive functions. Abbreviations: ACC, anterior cingulate cortex; Acb, nucleus accumbens; AMY, amygdala; BST, bed nucleus of stria terminalis; Hyp, hypothalamus; mPFC, medial prefrontal cortex; LM, lateral mammillary nucleus; MM, medial mammillary nucleus; RSP, retrosplenial cortex; VTA ventral tegmental area. Figure adapted from (O'Leary and Cryan, 2014).

1.1.5 Gamma oscillations

Gamma oscillations have been studied in many cortical regions and in the hippocampus and have been associated with many different processes and behaviours including visual processing (Gray and Singer, 1989), motor function (Pfurtscheller and Berghold, 1989; Pfurtscheller and Neuper, 1992; Pfurtscheller, Neuper and Kalcher, 1993; Crone *et al.*, 1998), olfactory function (Eeckman and Freeman, 1990) and memory and sensory processing (Gray and Singer, 1989; Traub *et al.*, 1996; Traub *et al.*, 1999). Gamma oscillations are abundant in most behavioural states but are particularly prevalent during primary sensory processing during wakefulness (Gray and Singer, 1989). Hippocampal gamma oscillations have been shown to be important in memory encoding (Newman *et*

al., 2013), working memory (Yamamoto *et al.*, 2014) and spatial navigation tasks in rodents (Bieri, Bobbitt and Colgin, 2014; Zheng *et al.*, 2016). The entorhinal cortex acts as an interface between neocortical regions and the dentate gyrus and cornu ammonuis (Witter *et al.*, 1989). Superficial neurons of the entorhinal cortex supply the main neocortical input to the hippocampus via the perforant pathway and therefore occupies a pivotal role in the process of gating information flow along the neo-archicortical axis. (Steward and Scoville, 1976; Witter and Groenewegen, 1984; Amaral and Witter, 1989) The entorhinal cortex provides inputs from several sensory regions and engages in a functional interplay of sensory information with the hippocampus (Fernandez *et al.*, 1999; Fell *et al.*, 2001). The entorhinal cortex has been shown to undergo pathologic changes that occur in disorders of memory function such as Alzheimer's disease (Hyman *et al.*, 1984; Braak and Braak, 1991). During rodent spatial learning tasks, entorhinal cortex and hippocampal gamma oscillations measured from the dentate gyrus synchronise (Fernandez-Ruiz *et al.*, 2021).

Gamma oscillations are generated in numerous ways involving different mechanisms and networks and have a frequency band encompassing a range from 25-150 Hz, with some studies categorising the frequency band into low (25-100 Hz) and high (100-150Hz) sub-bands (Herculano-Houzel *et al.*, 1999; Uhlhaas *et al.*, 2011). A variety of *in vitro* modelling systems have been developed to provide a pharmacological and mechanistic insight into gamma oscillations (Whittington, Traub and Jefferys, 1995; Fisahn *et al.*, 1998; Fisahn *et al.*, 2004). These use a combination of carbachol and the ionotropic glutamate receptor agonist kainic acid to elicit oscillations, although metabotropic glutamate receptor agonists and tetanic stimulations have also been used (Traub *et al.*, 1996; Macdonald *et al.*, 1998). There are two widely accepted models of gamma generation; the interneuron network gamma (ING) and pyramidal-interneuron network gamma (PING) models.

1.1.5.1 Interneuron network gamma (ING)

The ING model of gamma oscillations describes a mechanism in which interconnected interneurons can alone generate the population rhythm. The ING model relies on the interconnectivity of GABAergic interneurons, resulting in mutual and sustained inhibition amongst the intraneuronal network (Whittington *et al.*, 2000) and was originally shown by eliciting 40 Hz oscillations in hippocampus CA1 region (Traub *et al.*, 1996). The

network requires initial excitatory input to bring the intraneuronal population to a threshold level of activity, achieved by tonic (application of a metabotropic agonist in the presence of ionotropic glutamate receptor blockers), phasic (carbachol model) or both (the tetanus model) activation. In a two interneuron model, if one interneuron is depolarised, a high frequency of action potentials is generated in that interneuron. These action potentials produce a temporally summating train of inhibitory post synaptic potentials (IPSPs) in the non-depolarised interneuron. If both interneurons are concurrently depolarised then both fire synchronous action potentials at gamma frequencies, a consequence of the mutual inhibition between two neurons. An action potential in one interneuron will prevent an action potential in the other, and as the IPSP arrives, the most favourable time for second interneuron to fire is simultaneously with the first. Hence, a network of this configuration generates synchronous gamma frequency oscillations (Fig 1.5 A) (Whittington *et al.*, 2000). Once a population of interneurons are excited, the firing frequency of the population is governed by the time course of the mutual inhibition present from one neuron within the population to its synaptically connected neighbours (Whittington, Traub and Jefferys, 1995; Traub *et al.*, 1996; Wang and Buzsáki, 1996). It is important to note that the interneuron population must consist of neurons with intrinsic firing frequencies faster than the gamma range, which is common in depolarised inhibitory neurons in the CNS (Buhl *et al.*, 1994; Buhl *et al.*, 1996). The magnitude of the synaptic inhibition between interneurons is responsible for the frequency of ING. The frequency of gamma oscillations can be altered by a range of endogenous and exogenous agents that mediate the $GABA_A$ receptor-mediated IPSP. These agents include sedatives (Segal and Barker, 1984), anaesthetics (MacIver, Tanelian and Mody, 1991), hormones (Wan *et al.*, 1997), sex steroids (Teschemacher *et al.*, 1995) and other neuromodulators (Siarey, Andreasen and Lambert, 1995).

1.1.5.2 Pyramidal-interneuron network gamma (PING)

Early studies of gamma oscillations *in vivo* demonstrated networks composed of both excitatory and inhibitory connections (Leung, 1982; Jagadeesh, Gray and Ferster, 1992), therefore, to replicate the *in vivo* conditions of the brain, the reciprocal influence of excitatory neurons within the interneuron network activity was investigated. It was discovered that an oscillation is produced involving the interplay between excitatory pyramidal cells and interneurons, a concept known as pyramidal interneuron network gamma (PING) and occurs in situations where metabotropic receptor activation not only

generates ING but recruits pyramidal cells (Whittington *et al.*, 1997). In an intact local network, the activity of excitatory neurons will modify the behaviour of the interneuron network via feedback excitation of interneurons. Excitatory neurons recover from inhibition before inhibitory neurons which allows the pyramidal cells to fire EPSPs onto inhibitory cells before they become inhibited by another arriving IPSP, therefore the activity of the excitatory and inhibitory cell alternates. The pyramidal neurons excite the interneuronal population through glutamatergic neurotransmission via AMPA receptors. The subsequent neuronal output from interneurons inhibits other interneurons (as described in the ING mechanism) as well as the mutual inhibition of the locally connected pyramidal neurons (Fig 1.5 B). The time decay of the IPSP determines the frequency of the oscillation (Whittington *et al.*, 2011).

The involvement of excitatory neurons strengthens the network and makes it less vulnerable to loss of coherence when there is heterogeneity in driving forces (Traub *et al.*, 1996; Wang and Buzsáki, 1996; White *et al.*, 1998). Additionally, the involvement of excitatory pyramidal cells in the PING model also results in the ability of the network to synchronise over distances of up to 3.5mm, with spatially distinct regions being shown to oscillate together with little phase-lag (Whittington *et al.*, 1997; Ermentrout and Kopell, 1998).

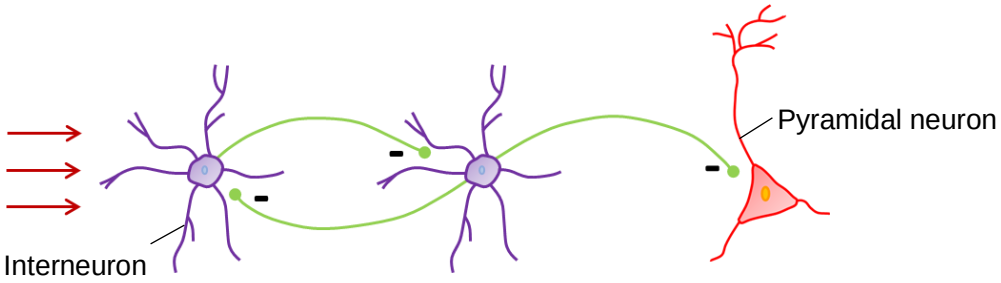
1.1.5.3 Persistent gamma oscillations

The PING model of oscillations provides mechanistic insight for the generation of gamma rhythms in the cortex, however experimentally it exists for brief periods of time following glutamate pressure injection and tetanic stimulation (Whittington *et al.*, 1997). PING involves the somatic spiking of pyramidal cells; however these have been observed to occur at 1-5 Hz during persistent gamma (Fisahn *et al.*, 1998; Chapman and Lacaille, 1999). Spiking at this frequency range is insufficient to provide the excitatory drive for a persistent rhythm, even with the convergence of multiple pyramidal cells onto single interneurons (Whittington and Traub, 2003). The mechanism underlying persistent gamma oscillations involves gap-junctional communication between axons of fast rhythmic bursting neurons (Hormuzdi *et al.*, 2001). High frequency oscillations (>90 Hz) are generated in the axonal plexus, spike multiple times per oscillation, and provide phasic input to interneurons required to generate persistent gamma oscillations (Fig 1.5 C) (Traub *et al.*, 2003; Cunningham *et al.*, 2004). Stable persistent gamma oscillations

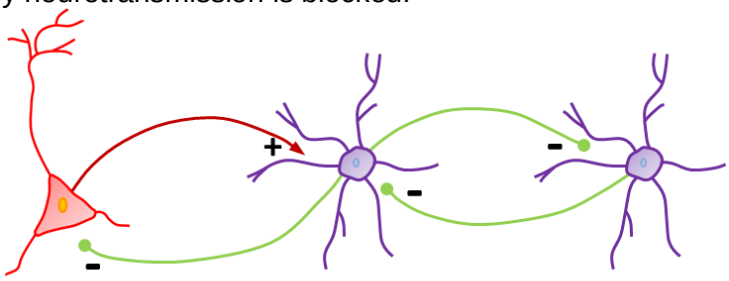
can be generated in acute rodent brain slices by bath application of a muscarinic and nicotinic acetylcholine receptor agonist, carbachol (Fisahn *et al.*, 1998). Carbachol has been shown to generate robust hippocampal gamma oscillations, however its induction of cortical gamma oscillations is less successful. In *in vitro* entorhinal cortex slices, carbachol has resulted in the generation of epileptiform activity (Dickson and Alonso, 1997; Gloveli *et al.*, 1999). Kainic acid, an exogenous ligand for the glutamate-sensitive excitatory kainite receptors, is more reliable at generating robust gamma oscillations in the entorhinal cortex. Kainic acid generates persistent gamma in medial entorhinal cortex in *in vitro* slices in manner like that seen in the cornu ammonis, whereby both are sensitive to $GABA_A$ and AMPA receptor blockade, barbiturates and reduction in gap junctional conductance (Cunningham *et al.*, 2003). Therefore, in this thesis, kainic acid was used to generate robust, persistent gamma oscillations simultaneously in the entorhinal cortex and CA3.

1.1.6 Theta oscillations

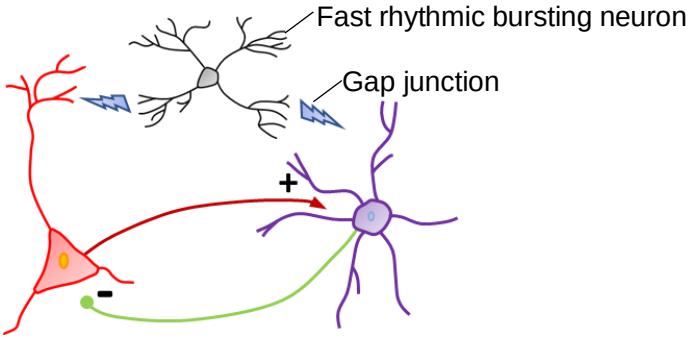
In addition to gamma rhythms, the hippocampus is also the main structure involved in the generation of the theta oscillation (4-12 Hz) which is linked with higher cognitive function (Green and Arduini, 1954). Theta arises from interactions between glutamatergic, cholinergic and GABAergic neurons of the medial septum, diagonal band of Broca neurons and the hippocampus (Colom *et al.*, 2005; Stumpf, Petsche and Gogolak, 1962; Mitchell *et al.*, 1982), but are also detected in the entorhinal cortex, somatosensory cortex and the prefrontal cortices (Alonso and García-Austt, 1987; Nuñez, de Andrés and García-Austt, 1991; Sirota *et al.*, 2008). The theta rhythm is associated with memory formation, particularly episodic memory (Kerrén *et al.*, 2018; Lega, Jacobs and Kahana, 2012) and spatial navigation (O'Keefe and Recce, 1993; Gupta *et al.*, 2012), and theta and gamma oscillations are important for separating encoding and retrieval states in the hippocampus (Colgin *et al.*, 2009; Cutsuridis, Cobb and Graham, 2010). Phase-amplitude coupling between theta and gamma oscillations increase during successful encoding (Lega *et al.*, 2016; Mormann *et al.*, 2005), and gamma-theta coupling is thought to be vital in learning and memory processes and proper cognitive functioning (Musaeus *et al.*, 2020). Loss of theta-gamma coupling is thought to contribute to neurodegenerative disorders such as Alzheimer's Disease (Zhang *et al.*, 2016; Goodman *et al.*, 2018; Bazzigaluppi *et al.*, 2018).



(A) Interneuron network gamma (ING). Gamma oscillations are generated by interconnected GABAergic interneurons. Initial activation through tonic or phasic activation causes depolarisation of an interneuron and subsequent firing of action potentials. This results in a train of inhibitory postsynaptic potentials (IPSPs) in nearby non-depolarised interneurons. If interneurons are concurrently depolarised, they fire synchronous action potentials at the gamma frequency. They can influence pyramidal cells, but excitatory neurotransmission is blocked.



(B) Pyramidal Interneuron network gamma (PING). Pyramidal cells provide the excitatory drive to the interneuronal population through glutamatergic neurotransmission via AMPA receptors. The subsequent neuronal output from interneurons inhibits other interneurons (as described in the ING mechanism) as well as the mutual inhibition of locally connected pyramidal neurones. The time decay of the IPSP determines the frequency of the oscillation.



(C) Persistent gamma oscillations. Pyramidal cells spike at a frequency too low to maintain persistent gamma oscillations. Fast rhythmic bursting neurons spike multiple times per oscillation and through gap-junctional communication, provide phasic input to pyramidal neurons and interneurons generating persistent oscillations at the gamma frequency.

Figure 1.5 Schematic representation of gamma generation through ING (A) and PING (B) mechanisms and persistent gamma oscillations (C). Figure adapted from Whittington *et al*., (2000) and Shin *et al*., (2011).

1.1.7 The hippocampus, aging and disease

Neuroplasticity is the ability of the brain structures to adapt in response to stimuli by reorganising its structure, function and connections, and can occur due to environmental changes, development, in support of learning or in response to disease (Cramer *et al.*, 2011; Kolb and Muhammad, 2014). The hippocampus has long been considered a classic example for the study of functional neuroplasticity, as many models of synaptic plasticity such as long term potentiation (LTP) and long-term depression (LTD) are thought to be fundamental to learning and memory (Bliss and Schoepfer, 2004; Pastalkova *et al.*, 2006). This high degree of plasticity is accompanied by the region's vulnerability to conditions such as neuroinflammation, neurodegeneration, ischemia, epilepsy and aging, suggesting that the intrinsic properties of hippocampal circuits and neurons that are critical for neuroplasticity, may also predispose the region to the metabolic injuries that occur in neurological and psychiatric diseases (Bartsch *et al.*, 2015; Bartsch and Wulff, 2015).

Aging is a natural biological process that is associated with physical and cognitive decline. With normal aging, cognitive decline can present as deficits in episodic memory, spatial learning, working memory and attention (Kausler, 1994). A number of behavioural experiments show that aged individuals exhibit impaired learning and memory performance compared to young counterparts in rodent models (Gage *et al.*, 1984; Mizumori, Lavoie and Kalyani, 1996; Shen *et al.*, 1997), primate models (Rapp, Kansky and Roberts, 1997) and human models (Newman and Kaszniak, 2000; Moffat, Zonderman and Resnick, 2001). The decline in memory performance during aging could be due in part to structural changes in the hippocampus with aging, (Bettio, Rajendran and Gil-Mohapel, 2017; Persson *et al.*, 2012), including an age-dependent reduction in hippocampal volume which has been observed in male and female rodents (Driscoll *et al.*, 2006; von Bohlen und Halbach and Unsicker, 2002) and in human subjects (Driscoll *et al.*, 2003; Schuff *et al.*, 1999). The decrease in hippocampal volume is thought to be correlated with hippocampal neurone loss during the aging process. A significant decrease in the number of NeuN-positive neurons and non-neuronal cells is reported in a rodent model of aging (Fu *et al.*, 2015), and a study of hippocampal neuron numbers in the human brain described a loss of cells in the subiculum and hilus of the hippocampus (West, 1993). Some controversy exists however, as other reports detect no significant association between hippocampal volume change with aging

(Maheswaran *et al.*, 2009; Spreng *et al.*, 2010). In rodents, aging is associated with impaired hippocampal LTP which is related to a decreased ability to consolidate long-term memory and has resulted in poor performances in hippocampal-dependent spatial memory tasks in rats (Lister and Barnes, 2009; Barnes and McNaughton, 1985; Barnes and McNaughton, 1980). Furthermore, a decrease in the number of hippocampal synapses and a reduction in the expression of synaptic proteins is thought to likely contribute to a reduction in hippocampal synaptic transmission with age and further contributing to age-related cognitive deficits (Wang *et al.*, 2007; Gureviciene, Gurevicius and Tanila, 2009).

In the hippocampal formation, the perforant path particularly shows sensitivity to age-related deterioration. In rats, perforant pathway synapses which interact with dentate gyrus granule neurons show a decrease in N-methyl-D-aspartate (NMDA) receptor-mediated responses, fewer synaptic contacts, and a reduction in LTP during aging (Barnes, Rao and Houston, 2000; Burke and Barnes, 2010). *In vivo* imaging studies in humans also demonstrate perforant pathway synaptic deterioration with aging, with the extent of the degeneration correlating with hippocampal-dependent memory deficits (Yassa, Muftuler and Stark, 2010; Burger, 2010).

1.1.7.1 Early aging pathologies

Most models of hippocampal function and aging compare young subjects with aged, with the experiments forementioned comparing age groups that are 2-3 months old with 18-30 months old in rodent models and 18-35 year olds with 60+ year olds in human models. Rodents are often used as a modelling of aging due to their short lifespans, and in rodent aging literature, a variety of animal strains and respective ages are often utilized. Most aging research is conducted in C57/BL6, BALB/c or CBA mice, and generally mice between 2-3 months old are classified as young rodents, whilst aged are often classified as 18+ months old (Radulescu *et al.*, 2021; Brubaker, Palmer and Kovacs, 2011). These studies have revealed how a variety of physiological, functional and behavioural changes are affected in old age, however aging is defined as a progressive deterioration of physiological function with increasing age (Galloway, 1993), yet there is very little literature examining whether physiological aging is also progressive. The biological onset of neurodegenerative disorders precedes prodromal clinical symptoms such as mild cognitive impairment by several years to decades (Jack and Holtzman, 2013), and

understanding the very earliest stages of senescence may allow for the development of targeted approaches that can slow the negative impact of aging or even rejuvenate function (Wyss-Coray, 2016; Castellano *et al.*, 2017).

More recent work has documented how hippocampal function is affected in middle age. Hippocampal-dependent spatial memory in humans has been shown to gradually decline between young (18-25 year olds), middle age (40-55 year olds) and the elderly (60 + years old) (Williams *et al.*, 2019), which is associated with clinical onset of Alzheimer's Disease (Ritchie *et al.*, 2018), and spatial visualisation, memory, and reasoning is reported to decline chronologically with aging human subjects (Salthouse, 2009). Additionally, neural circuitry has been documented to be become disrupted in middle age, with reduced dorsal hippocampal activity reported between 2-3 month old mice and 10-14 month old mice (Schreurs, Sabanov and Balschun, 2017) and neurogenesis markers decline when rodents age from 4 months to 12 months in all hippocampal subregions (Hattiangady *et al.*, 2005). Aging from middle age to old age also shows changes in brain physiology and behaviour. A reduction in neurone number in the olfactory bulb and hippocampus is observed between 11-12 month old mice and 25-31 month old mice (Shen *et al.*, 1997), and old mice exhibit spatial navigation impairment which was associated with a reduction in dorsal hippocampal theta frequency (Shen *et al.*, 1997). Spatial navigation memory is also shown to be reduced between middle aged (10-12 months old) and old mice (18-20 month old mice), with more pronounced cognitive decline observed in female mice compared to males (Benice *et al.*, 2006).

These experiments suggest that hippocampal function and cognition could decline progressively with aging, however, there appears to be subminimal investigation into whether hippocampal function is affected in early aging, aging from young (2-3 months old) to adulthood (6-8 months of old) for example, and therefore the trajectory for age-dependent decrease in hippocampal function is not fully clear.

1.2 The innate immune system

1.2.1 Innate immune function

1.2.1.1 Proinflammatory and anti-inflammatory mechanisms

The major role of the immune system is to maintain homeostasis in response to changes in internal and external environments, with the ideal immune response eliminating pathogens and re-establishing homeostasis without causing excessive damage to healthy cells and tissues (Paludan *et al.*, 2021). The innate immune system is one of the two main immunity strategies, with the other being the adaptive immune system. The innate immune system is a non-specific first line of defence immune response which acts to immediately identify and remove foreign pathogens through hormonal and cellular defences (Beutler, 2004). The first step of the innate pro-inflammatory cascade involves recognition of infection or damage which is typically achieved by pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), intracellular nucleotide binding domain receptors (NOD-like receptors) and leucine-rich-repeat-containing receptors (NLRs) (Lange *et al.*, 2011; Proell *et al.*, 2008; Roach *et al.*, 2005). PRRs detect pathogen-associated molecular patterns (PAMPs), which include fungal and bacterial cell-wall components and viral nucleic acids (Medzhitov, 2007), in addition to damage-associated molecular patterns (DAMPs), which are molecules that signal damage or necrosis (Roh and Sohn, 2018). The detection of PAMPs and DAMPs by PRRs leads to the activation of a transcription factor called nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), which translocate to the nucleus where transcription is upregulated through binding to target inflammatory genes (Ashley, Weil and Nelson, 2012). Transcription and translation of genes leads to the expression of proinflammatory cytokines, which facilitate the recruitment of effector cells (such as monocytes and neutrophils) to the sites of disturbance to remove harmful agents (Iwasaki and Medzhitov, 2015). These mechanisms lead to symptoms of local inflammation which includes heat, swelling, pain, redness, and loss of function. The effector functions of inflammation are further regulated by the adaptive immune system, which acts to create immunological memory after an initial response to a particular pathogen which results in an enhanced response to future encounters to the same pathogen (Bluestone and Abbas, 2003).

The last phase of acute inflammation is a process called resolution, which acts to suppress innate/adaptive immune responses, blocks further neutrophil recruitment, and enhances infiltration of monocytes important for wound healing (Ashley, Weil and Nelson, 2012) (Ashley, Weil and Nelson, 2012). This process is crucial for limiting damage to the host, and often initiates in the first few hours after an inflammatory response begins (Serhan and Savill, 2005). After entering tissues, granulocytes (such as neutrophils) promote the switch of inflammatory mediators arachidonic acid-derived prostaglandins and leukotrienes (which regulate early events in the inflammatory response) to lipoxins, which serve as stop signals by blocking further recruitment of neutrophils, eosinophils, basophils and mast cells from post-capillary venules (Samuelsson *et al.*, 1987; Serhan *et al.*, 1995). Neutrophil recruitment ceases and programmed cell death by apoptosis is engaged (via macrophages), resulting in neutrophil clearance and release of anti-inflammatory cytokines which supress proinflammatory signalling from TLRs (Byrne and Reen, 2002). Macrophages are finally cleared through lymphatics (Serhan and Savill, 2005).

Dysregulation of pro- and anti-inflammatory mechanisms cause marked tissue damage, especially during prolonged periods of excessive inflammation, especially in organs and tissues that are particularly sensitive to immune-mediated change such as the brain (Paludan *et al.*, 2021; van der Poll *et al.*, 2017).

1.2.1.2 Acute and chronic inflammatory response

Inflammation and its sequelae vary spatially and temporally (Medzhitov, Schneider and Soares, 2012). Inflammation usually begins in a localised area in response to trauma, however depending on the severity of the trauma/infection, can spread rapidly to the periphery and trigger a systemic response that can lead to organ injury (Seeley, Matthay and Wolters, 2012) (Seeley, Matthay and Wolters, 2012). Systemic inflammation is triggered by proinflammatory cytokines, which are released into the circulation and activate fevers and sickness behaviours in the brain in addition to acute protein secretion from the liver (Cunningham *et al.*, 2009). The time course of inflammation can also incur a detrimental effect on overall health. Acute inflammation refers to a short-term process that is initiated immediately upon injury/infection and acts to conduct tissue repair and restore functionality, lasting minutes, hours or only a few days (Chen *et al.*, 2018). Inflammatory mediators are short-lived and are degraded quickly in tissue (Kumar *et al.*,

2004). Chronic inflammation lasts for several months to years and is referred to as persistent, low-grade inflammation and is associated with a variety of cardiovascular, metabolic, and neurodegenerative diseases, as well as stroke and myocardial infarction (Hotamisligil, 2006) and has been linked to age-associated disease (Finch and Crimmins, 2004).

1.2.2 The innate immune system and aging

There is a plethora of evidence that the immune system becomes deregulated with aging. It is well documented that adaptive immune function decreases with age, with the most prominent features of immunosenescence including a decrease in the number of lymphocytes as a result of a substantial decrease in the development and function of T cells and B cells (Linton and Dorshkind, 2004; Allman and Miller, 2005). The innate immune system is also affected by aging, with studies in aged mice (older than 20 months) and humans (over the age of 65) showing that activation of the aged innate immune system results in dysregulated inflammation (Gomez *et al.*, 2007; Bruunsgaard *et al.*, 2003; Fulop *et al.*, 2004). This dysregulation involves elevated levels of basal inflammation, particularly in humans, and an impaired ability to conduct efficient immune responses to newly encountered pathogens or vaccine antigens (Shaw, Goldstein and Montgomery, 2013). A mounting body of evidence from human studies indicates that older adults have elevated levels of pro-inflammatory cytokines and clotting factors (Bruunsgaard *et al.*, 1999; Fagiolo *et al.*, 1993; Mari *et al.*, 1995), and in general the innate immune system switches to a pro-inflammatory state in aged individuals (Franceschi *et al.*, 2000). The reason for elevated levels of pro-inflammatory cytokines with aging is unknown, however aging is associated with elevated levels of PRR ligands (PAMPS and DAMPs), arising through chronic viral infection or from cell damage, and these are thought to contribute to the downstream proinflammatory state (Pawelec *et al.*, 2012; Coppe *et al.*, 2010). In addition, changes in endocrine function and hormones, including those arising from alterations in adipose tissue are also thought to be responsible for age-dependent immune dysregulation (Garg *et al.*, 2014; Montgomery and Shaw, 2015). This dysfunction and dysregulation of the immune system can also lead to various immune-related diseases. Evidence suggests that low-grade chronic inflammation seen with aging contributes to diseases such as atherosclerosis, diabetes, lung disease and various neurodegenerative disorders including Alzheimer's disease,

Parkinson's disease and amyotrophic lateral sclerosis (Lathe, Sapronova and Kotelevtsev, 2014; McGeer and McGeer, 2004).

1.2.3 Cytokines

The innate and adaptive immune responses are co-ordinated by small, secreted proteins - the cytokines, which are produced by a number of cell types in response to invading pathogens (Zhang and An, 2007). They act to simulate, recruit, and proliferate immune cells, as well as mediate immune cell differentiation and survival (Foster, 2001). Cytokine synthesis and secretion is under tight regulatory control (Kany, Vollrath and Relja, 2019); once cytokines are released, their half-life is relatively short, which limits their biological activity (Whiteside, 1994). Cytokines exert their functions through a number of mechanisms: acting on cells that secrete them (autocrine action), on nearby cells (paracrine function) or on distant cells (endocrine function) (Zhang and An, 2007). Most cytokine activity occurs at a local level, where they act in a paracrine fashion to exert their effects by diffusion or cell-to-cell contact. Cytokines can also leave their local environment, enter circulation and interact with different organ systems and alter host physiology (Arango Duque and Descoteaux, 2014). It is common for different cell types to secrete the same cytokine, and for a single cytokine to act on several different cell types (Ozaki and Leonard, 2002). In addition, cytokines are also redundant in their activity, where multiple cytokines exert similar actions, and are often produced in a cascade where one cytokine stimulates its target cells to make additional cytokines (Yadav and Sarvetnick, 2003; Sadik and Luster, 2012). Cytokine action and cross-talk is highly complex, an overview of the proinflammatory and anti-inflammatory cytokine network is displayed in figure 1.6.

The cytokine signalling pathway is further complicated by biased signalling, also known as functional selectivity, where a cytokine binding to a receptor preferentially activates one signalling pathway out of several available pathways. This scenario can occur due to ligand bias, receptor bias, or cell/tissue bias. Ligand bias describes a scenario where different ligands bind to the same receptor, but induce diverse responses (Förster, Davalos-Misslitz and Rot, 2008). Receptor bias refers to the case where the same ligand induces different responses on different receptors, and cell/tissue bias describes where a ligand for a given receptor activates different pathways in a tissue/cell specific manner (Kenakin, 2012; Steen *et al.*, 2014).

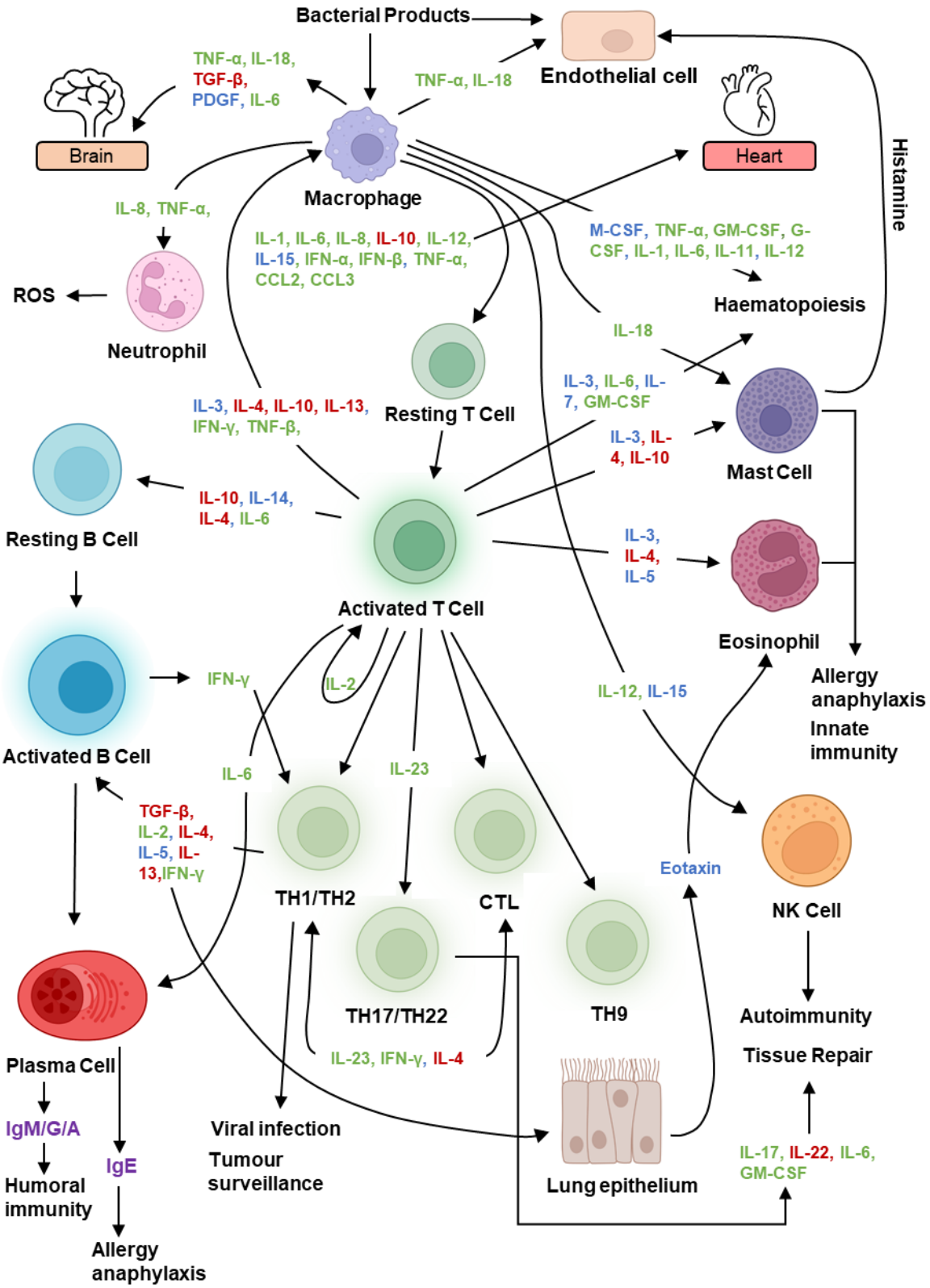


Figure 1.6 Cytokine network. Immune cells communicate via cytokines, which control cell proliferation, differentiation, and function. Proinflammatory cytokines (as shown in green) are crucial for coordinating immune responses and play an important role in modulating the immune system. Anti-inflammatory cytokines (as shown in red), regulate the proinflammatory cytokines response, whilst some cytokines are involved in promoting cell maturation and growth (as shown in blue). Figure adapted from Zhang 2007.

1.2.4 Chemokines

Chemokines are chemotactic cytokines that act to control cell migration and cell positioning throughout development, homeostasis, and inflammation and are critical for immune system function (Sokol and Luster, 2015). Chemokines act to guide leukocytes to sites of infection and inflammation and coordinate interactions between immune cells (Le Thuc *et al.*, 2015). They are small 8 - 14kDa protein ligands, and approximately 50 different chemokines exist in humans and mice, which are systematically categorised into four groups based on their positioning of their cystine residues: XC, CC, CXC and CX3C, with the largest family being the CC chemokines (Charo and Ransohoff, 2006). Chemokines act to direct leukocyte extravasation from blood and lymph into surrounding tissues, guiding leukocyte migration through complex environments such as the extracellular matrix (Strell and Entschladen, 2008). Effector cell navigation through such complex and dynamic environments is poorly understood (Quast *et al.*, 2022). It is proposed that leukocyte migration is controlled by chemokine concentration gradients, where lymphocytes expressing chemokine cognate receptors migrate up the gradient towards areas of high chemokine concentration, using spatial and temporal cues, that allows cells to orientate themselves and maintain their direction (Devreotes and Zigmond, 1988; Moore, Brook and Nibbs, 2018). Chemokine gradients are dynamic and depend on numerous unified physical and biological processes such as chemokine production rate, diffusion and fluid flow, in addition to the nature of the extracellular matrix (Schumann *et al.*, 2010; Patel *et al.*, 2001; de Paz *et al.*, 2007). More recent evidence suggests that effector cells require gradients with globally rising concentrations for persistent directional migration, rather than temporally stable gradients (Minton, 2018).

Chemokines exert their functions by binding to a group of approximately twenty rhodopsin-like seven-transmembrane-spanning, G-protein coupled cell surface receptors, also referred to as typical chemokine receptors (Rostene, Kitabgi and Melik-Parsadaniantz, 2007), which act to convert extracellular signals into intracellular responses. G-proteins exist as a heterotrimer composed of three distinct subunits. When the molecule GDP is bound to the G-protein subunit, the G-protein is in an inactive state. Following binding of the chemokine ligand, chemokine receptors associate with G-proteins, allowing the exchange of GDP for GTP, and the dissociation of the different G protein subunits. The Gα subunit activates the enzyme Phospholipase C (PLC), that is associated with the cell membrane. PLC cleaves Phosphatidylinositol (4,5)-

bisphosphate (PIP2) to form two second messenger molecules inositol triphosphate (IP3) and diacylglycerol (DAG); DAG activates the enzyme protein kinase C (PKC), and IP3 triggers the release of calcium from intracellular stores. These events promote many signalling cascades, and initiates a cellular response (Murdoch and Finn, 2000).

Chemokine receptors can be broadly classified into two classes: constitutive, which are involved in basal trafficking and development; or inflammatory, which are involved in activation of immune responses (Dotan *et al.*, 2010). Notably, chemokine receptors are highly dynamic structures and signalling efficiency largely depends on the discrete contact with the ligand (Corbisier *et al.*, 2015). However, this system is complex due to the promiscuity of both chemokines and chemokine receptors, and few receptors bind to a single ligand (Proudfoot, 2002; Hughes and Nibbs, 2018), as summarised in figure 1.7. Combined with biased signalling and allosteric modulation of receptor activation (Steen *et al.*, 2014), dysregulation of this tightly controlled system can result in downstream, cumulative disruption which can result in pathogenic physiology (Ferrucci and Fabbri, 2018).

Increases in the major pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumour necrosis factor-α (TNFα) (Franceschi *et al.*, 2000) and peripheral proinflammatory chemokines CCL2, CCL3, CCL5 and CXCL13 (Inadera *et al.*, 1999; Pulsatelli *et al.*, 2000; Gerli *et al.*, 2000; Kurosawa *et al.*, 2021) contribute to the phenomenon of “inflamm-aging” in healthy individuals and the pathology of age-related diseases such as Parkinson’s Disease, Alzheimer’s disease, cardiovascular disease, diabetes, chronic kidney disease, anaemia, osteoporosis and rheumatoid arthritis (Franceschi and Campisi, 2014; Lai *et al.*, 2017; Ruparelia *et al.*, 2017; Odegaard *et al.*, 2016; Amdur *et al.*, 2016; Ferrucci *et al.*, 2010). Therefore, modulating inflammation is a promising strategy not only to prevent the onset of age-related inflammatory diseases, but also to slow the decline of health that occurs with aging (Ferrucci and Fabbri, 2018).

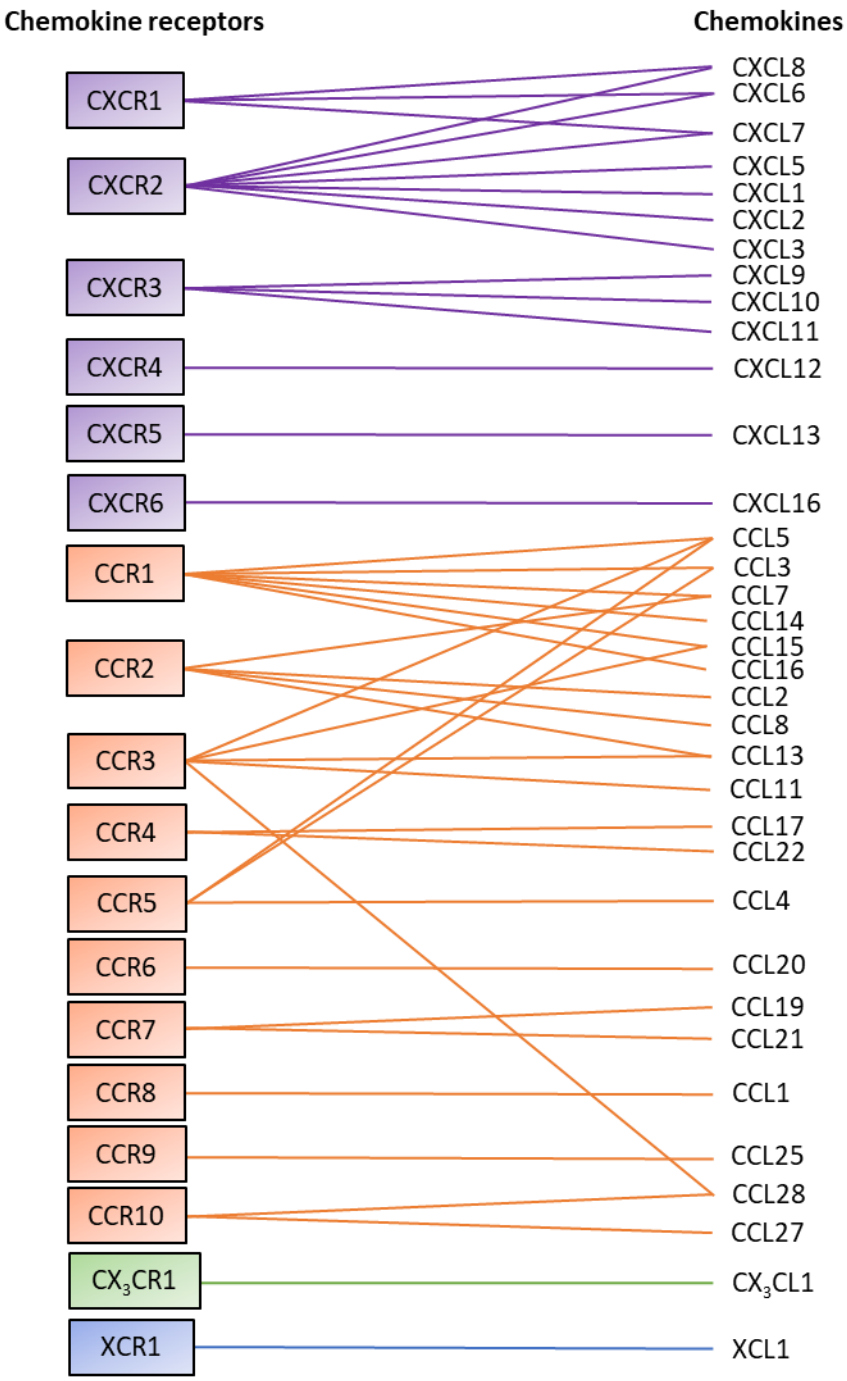


Figure 1.7 Chemokine receptors and their respective ligands. Chemokines are divided into 4 subgroups based on the spacing of N-terminal cysteine residues: C (depicted in blue), CC (orange), CXC (purple) and CX_3C (green). The pairing of chemokines to their receptors present confirmed interactions carried out by receptor binding assays. Figure adapted from (Proudfoot, 2002; Wells *et al.*, 2006).

1.2.5 Neuroinflammation

The CNS has its own regulatory immune responses, often referred to as the neuroimmune system, and is comprised of microglia, astrocytes, mast cells, ependymal cells and neurons (Tchessalova, Posillico and Tronson, 2018). Although this system lacks lymphatics, and uniquely prevents free diffusion of molecular and cellular

components by the blood brain barrier (BBB) (Ousman and Kubes, 2012), the brain's inflammatory response system is made up of complex processes that detect and eliminate mediators of infection and damage of neuronal tissue (Chen, Zhang and Huang, 2016).

1.2.5.1 Communication between systemic and CNS inflammation

The parenchyma of the CNS was originally considered an "immunologically privileged site" because the BBB was thought to prevent entry and exit of periphery molecules (Harris *et al.*, 2014). This led to CNS inflammation being viewed in isolation and independent of systemic inflammation (Holmes, 2013). However, it is now clear that systemic, peripheral inflammation and the CNS communicate and influence one another (Chambers, Cohen and Perlman, 1993). The concept of communication between systemic inflammation to the brain is supported by a vast number of *in vitro* and clinical studies. Direct activation of the peripheral immune system by the endotoxin lipopolysaccharide (LPS), or with one or more pro-inflammatory cytokines, gives rise to several sickness behaviour symptoms in rodents including fever, loss of appetite and decreased activity (Dantzer *et al.*, 1999; Dantzer *et al.*, 2000; Konsman, Parnet and Dantzer, 2002; Sparkman *et al.*, 2006; Wong *et al.*, 1997). In addition, in humans, sepsis is associated with sickness behaviour symptoms including lethargy, decreased attention and concentration, and is associated with raised peripheral cytokines and increased microglial activation (Bone, 1991; Sharshar *et al.*, 2004; Lemstra *et al.*, 2007). Even low level infection initiated through low doses of *Salmonella equi* and *Escherichia coli* LPS have had significant effects on memory and depression scores and are associated with marked increases in circulating TNF and IL-6 (Cohen *et al.*, 2003; Reichenberg *et al.*, 2001; Krabbe *et al.*, 2005).

Communication between systemic and CNS inflammatory signals in mice and humans has been proposed to occur through several different mechanisms. The first proposed mechanism involves the direct entry of immune cells such as monocytes and bone marrow-derived microglia from the periphery into the brain. Bone-marrow derived microglia have been reported to infiltrate into the brain parenchyma from the blood during animal models of brain injury, chronic stress and disease including amyotrophic lateral sclerosis (ALS), multiple sclerosis, experimental autoimmune encephalomyelitis and Alzheimer's disease (Ataka *et al.*, 2013; Beers *et al.*, 2006; Ajami *et al.*, 2011; Davoust

et al., 2008; Sharma *et al.*, 2010). Peripheral and CNS communication also occurs via stimulation of peripheral nerves by cytokines and prostaglandin; low levels of cytokines/prostaglandins introduced directly into the abdominal cavity has been shown to directly activate vagal afferents, which signal to the medulla oblongata and are relayed to the hypothalamus, influencing brain function (Dantzer *et al.*, 2000; Romanovsky, 2004; Székely *et al.*, 2000; Ek *et al.*, 1998). A third mechanism involves direct action of LPS/pro-inflammatory cytokines at sites lacking a BBB. For example, a number of specialised neural regions exist that are in contact with the cerebroventricular system that contain a lack of tight-junctions between the capillary endothelial cells, such as the median eminence, the area postrema and the organum vasculosum of the lamina terminalis (Blatteis *et al.*, 1983). LPS and pro-inflammatory cytokines stimulate TNF production from perivascular and microglial macrophages, which then activate the proinflammatory transcription factor NF-kB in these areas of reduced BBB (Nadeau and Rivest, 2000; Breder *et al.*, 1994; Quan *et al.*, 1999). Systemic and central inflammation are also thought to communicate via direct action of LPS/pro-inflammatory cytokines on BBB vascular cells. This direct action activates intracellular signalling cascades via increased expression of the enzyme cyclooxygenase-2 (COX-2) in vascular endothelial cells and perivascular macrophages, that results in the synthesis of prostaglandins (Turini and DuBois, 2002). Animals lacking COX-2 fail to produce a fever response to either systemic LPS or IL-1β, and COX-2 inhibitor use blocks the fever response (Matsumura and Kobayashi, 2004; Yamagata *et al.*, 2001). The final proposed mechanism of action involves the active transport, or “shuttling”, of proinflammatory chemotactic cytokines across the BBB via atypical non-signalling receptors, which has been demonstrated *in vitro* (Minten et al., 2014).

1.2.5.2 Microglia function

Microglia are the resident macrophages of the CNS and act as the primary immune cell in the brain and spinal cord (Lawson *et al.*, 1990). Microglia have contact with surrounding cells and mediate a variety of functions including immune surveillance (Nimmerjahn, Kirchhoff and Helmchen, 2005), synaptic regulation (Wake *et al.*, 2009; Tremblay, Lowery and Majewska, 2010) and regulate neuronal activity and neurogenesis (Nakanishi and Wu, 2009). In the healthy CNS, microglia act as maintenance cells, have a ramified morphology and provide efficient spatial coverage throughout the CNS (Wong, 2013; Kreutzberg, 1996). However during an immune response, microglia undergo

activation in response to pro-inflammatory stimuli, such as in response to pro-inflammatory cytokines (Ginhoux *et al.*, 2013). Activated microglia become de-ramified, express larger cell bodies, shorter and thicker processes with extensive branching and act to phagocytose foreign material (Fernandez-Arjona *et al.*, 2017; Davis, Foster and Thomas, 1994). In addition to this phagocytic role, microglia are involved in inflammatory feedback signalling by releasing pro-inflammatory cytokines and chemokines which activate nearby microglia and other effector cells (Li and Barres, 2018; Kettenmann *et al.*, 2011).

Microglia also undergo several morphological and proliferative changes with aging. Microglia cell number increases in the hippocampus and visual and auditory cortices with aging (Mouton *et al.*, 2002; Tremblay *et al.*, 2012). During aging microglia also become de-ramified, with smaller dendritic arbors, increased branching and longer process lengths (Damani *et al.*, 2011), highlighting that aging also enhances microglia pro-inflammatory state, (Wong, 2013). Dysfunction of microglia inflammatory signalling has been associated with chronic inflammation and the contribution to many neurological and neurodegenerative conditions including Alzheimer's disease (Haga, Akai and Ishii, 1989; Itagaki *et al.*, 1989), Parkinson's disease (McGeer *et al.*, 1988), multiple sclerosis (Raine, 1994), amyotrophic lateral sclerosis (Engelhardt and Appel, 1990) and human immunodeficiency virus (Dickson *et al.*, 1993).

1.2.5.3 Astrocyte function

Astrocytes represent the largest group of glial cells and are responsible for a variety of functions including synapse development, neurotransmitter homeostasis, glycogen storage and the formation and maintenance of the BBB (Sofroniew and Vinters, 2010; Palmer and Ousman, 2018). In addition to their homeostatic functions, astrocytes are involved in CNS inflammation by responding to inflammatory signals and promoting inflammation, which makes them important components in the pathophysiology of neurogenerative diseases such as multiple sclerosis, Alzheimer's Disease, Parkinson's Disease and amyotrophic lateral sclerosis (Di Giorgio *et al.*, 2007; Habib *et al.*, 2020; Wheeler and Quintana, 2019; Solano *et al.*, 2008). Astrocytes in human and mice express a variety of PRRs and can recognise PAMPS through several TLRs and NOD-like receptors, which consequently triggers signalling cascades that promote pathogen clearance. For example, astrocytes stimulated with LPS produce and release a number

of cytokines including IL-1β, IL-6 and TNF and chemokines including CCL2 and CXCL1 (Geyer, Jacobs and Hsu, 2019; Choi *et al.*, 2014).

Astrocytes have also been shown to be affected by aging by showing a reduced ability to appropriately maintain a healthy CNS environment (Palmer and Ousman, 2018). With age in humans and rodents, astrocyte phenotypes alter from long and slender processes to short and thick processes, in addition to alterations in astrocyte density (Cerbai *et al.*, 2012; Jyothi *et al.*, 2015; Amenta *et al.*, 1998). With aging, astrocyte density decreases in the retina, whilst density has been observed to increase in the human cortex and hypothalamus (Mansour *et al.*, 2008; Hansen, Armstrong and Terry, 1987; Wang *et al.*, 2006). Reports on aging astrocytes have shown that glial fibrillary acidic protein (GFAP) expression increases, and as GFAP expression is a common feature of activated astrocytes, these findings suggest that astrocytes become reactive with age (Wu, Zhang and Yew, 2005; Clarke *et al.*, 2018; Liddelow *et al.*, 2017). In aging astrocytes, upregulation of pro-inflammatory genes has been reported, implying that astrocytes could contribute to the inflammatory state characteristic with aging (Mangold *et al.*, 2017; Palmer and Ousman, 2018). Another characteristic of aged astrocytes is an increase in cytokine production (Clarke *et al.*, 2018). Aged astrocytes produce increased amounts of CXCL10 and CXCL5, which attracts peripheral immune cells and aids T-cell adhesion to endothelial cells, and neutrophil recruitment, respectively. In addition to chemotactic and survival effects on immune cells via cytokine release, aged cortical astrocytes may negatively impact CNS health due to a decline in the production of metabolic and tropic factors such as ATP and neurotrophins. As ATP is involved in regulating neuronal activity through synaptic and tonic inhibition, a decline in ATP may lead to impaired synaptic plasticity and contribute to cognitive decline (Lalo, Rasooli-Nejad and Pankratov, 2014).

1.2.5.4 Neuroinflammation in aging and disease pathogenesis

It is recognised that the nervous, endocrine and immune systems are intimately linked and are interdependent, and it is widely accepted that a neuro-immune endocrine system allows for the preservation of homeostasis and overall health (De la Fuente and Miquel, 2009). Even though the neuroimmune system and peripheral immune system are structurally distinct, certain peripheral immune cells are able to cross the blood brain barrier to carry out immune functions in the brain, highlighting that both the innate and

peripheral immune system can both contribute to neuroinflammation (Gutierrez, Banks and Kastin, 1993).

Neuroinflammation is a natural response in the CNS that can be triggered by altered cerebral environment secondary to infection, trauma, epilepsy, environmental factors, autoimmunity and normal healthy aging (Gendelman, 2002), The classic role of inflammation is to remove or inactivate noxious agents and inhibit and reverse their detrimental effects (Kielian, 2014), yet inflammation in brain tissue is detected and associated with a number of neurodegenerative, neuropsychiatric and viral illnesses (Garden, 2002; Miller, Maletic and Raison, 2009; Heneka *et al.*, 2015). Particularly, neuroinflammation plays a significant role in the pathogenesis of severe diseases related to cognitive function and memory in later life such as Alzheimer's disease (West *et al.*, 1994; Lai *et al.*, 2017) and Parkinson's Disease (Hirsch, Vyas and Hunot, 2012) .

The brain is acutely sensitive to inflammatory molecules, and the balance between anti- and pro-inflammatory influencers greatly affects neuronal function, and this balance has been reported to become disrupted in normal aging. Genes associated with inflammation and oxidative stress are upregulated in aged rodents (Prolla, 2002), along with an increase in activation of microglia and astrocytes (Godbout *et al.*, 2005; Lyons *et al.*, 2009). It is widely accepted that the number of T-cells (which are important components of general increase of pro-inflammatory state) are low in a healthy brain, and numbers dramatically increase in response to immunological challenge (Bailey *et al.*, 2006). An increase in T-cells have also been reported to correspond with an increase in age (Stichel and Luebbert, 2007) and the passage of T-cells into the brain is facilitated by CCL2 and CCL3 which both increase in concentration with age (Kumagai *et al.*, 2007). The upregulation of cytokines IL-1β, IL-6, IL-18 and TNF-α with aging is also detected in the brain (Li *et al.*, 2009; Campuzano *et al.*, 2009; Martin *et al.*, 2002; Sue and Griffin, 2006), and an increase in pro-inflammatory mediators in the brain can affect behaviour (Lynch, 2010). For example, IL-1β has been frequently studied and an increase in its concentration in the hippocampus has been shown to impair hippocampal-dependent learning and spatial memory tasks (Gibertini *et al.*, 1995; Barrientos *et al.*, 2002) and reduction in exploratory behaviour, social exploration, social interaction and impacts on feeding and sleep have also been documented (Goshen and Yirmiya, 2009).

1.2.5.5 Neuro-immune changes with early aging

Similarly to the studies examining age-related pathologies as discussed in 1.1.7.1, the investigation into inflammatory changes during aging usually compare young cohorts with aged. The literature mentioned in section 1.2.3.2 investigate aged human participants ranging between 63-79 years old and mice models 18-30 months old, and few studies have investigated whether inflammation in the circulatory system and brain is upregulated in mid-aged individuals. Some evidence suggests that neuroinflammation is also disrupted during earlier aging, as microglia from middle aged rodents (9 – 10 months old) have greater expression of proinflammatory cytokines than young rodents (2 months old) (Nikodemova *et al.*, 2016), and greater microglia proliferation in mid aged humans (30 – 54 years old) was associated with poorer spatial reasoning, learning and memory and executive function (Marsland *et al.*, 2015). However, there is a gap in the current literature exploring if neuro-immune function changes in early aging. This is an important avenue of exploration as inflammation in the brain has been directly related to a range of age-related neurodegenerative diseases (West *et al.*, 1994; Lai *et al.*, 2017; Morales *et al.*, 2014; Hirsch, Vyas and Hunot, 2012), however the age at which these pathological mechanisms begin is unknown. Identifying if age-related immune pathologies begin at a certain age could provide a crucial point for therapeutic intervention to help prevent the cascade of pro-inflammatory mechanisms which lead to neurodegeneration.

1.2.6 CCL2

CCL2, also named as monocyte chemoattractant protein-1 (MCP-1), is a chemokine considered to be a marker of inflammaging, with numerous studies demonstrating plasma levels of CCL2 correlate with chronological age in humans and mice (Pinke *et al.*, 2013; Sesso *et al.*, 2015; Chiao *et al.*, 2011) and circulating levels are increased in patients with renal disease, cardiovascular disease and Alzheimer's disease (Akdogan *et al.*, 2015; Deo *et al.*, 2004; Bettcher *et al.*, 2016). CCL2 is produced by several cell types including endothelial, epithelial, myocytes, monocytes and microglial cells, either in a constitutive manner or in response to various stimulants such as oxidative stress and growth factors (Deshmane *et al.*, 2009). CCL2 exerts its biological effects by binding to its cognate receptor CC motif receptor 2 (CCR2), which induces monocytes to exit the bloodstream to act as tissue macrophages in response to inflammatory signalling (Deshmane *et al.*, 2009; Yousefzadeh *et al.*, 2018). CCR2 is one of the most prominent

chemokine receptors associated with CNS neuroinflammatory processes (Banisadr *et al.*, 2002; Sokolova *et al.*, 2009). CCL2 in the CNS arises either from blood-brain barrier diffusion from the peripheral immune system or via microglia production (Duan *et al.*, 2018; Cherry *et al.*, 2020), and its expression levels are elevated in astrocytes and microglia under pathological conditions (Zhang *et al.*, 2017). Elevated CCL2 is an important mediator of the neuroinflammatory responses in ischemic brain injury (Minami and Satoh, 2003), brain trauma (Glabinski *et al.*, 1996) and neurodegenerative disease including multiple sclerosis (Simpson *et al.*, 1998; Mahad and Ransohoff, 2003), Alzheimer's Disease (Ishizuka *et al.*, 1997; Bettcher *et al.*, 2016) and human immunodeficiency virus type-1 (HIV-1) associated dementia (Zink *et al.*, 2001; Dhillon *et al.*, 2008). Animal studies show that CCL2 and CCR2 are constitutively expressed in CNS neurons, localised in brain regions such as the hypothalamus, substantia nigra, cerebellum, cerebral cortex and hippocampus at the protein level via immunohistochemical staining and ELISAs, and at the mRNA level identified by reverse transcription PCR (Gosselin *et al.*, 2005; Banisadr *et al.*, 2005a; Banisadr *et al.*, 2005b). CCL2 has also been described to function as a neuromodulator in the CNS (Jung *et al.*, 2008; Melik-Parsadaniantz and Rostene, 2008) as its expression is co-localised with neurotransmitters (Banisadr *et al.*, 2005b) and cell membrane depolarisation can induce calcium-dependent release of CCL2 (Zhou *et al.*, 2011). CCL2 has been described to enhance neuronal excitability and synaptic transmission via presynaptic mechanisms in the hippocampus. An *in vitro* electrophysiology study on rat hippocampal slices using whole-cell patch camp technique revealed that CCL2 depolarised membrane potential, increased spike firing in CA1 neurons, produced an increase of excitatory post synaptic currents in Schaffer-collateral fibres to CA1 synapses and increased the frequency of spontaneous excitatory events (Zhou *et al.*, 2011). CCR2 and CCL2 are also increased in the hippocampus following neuroinflammation induced by status epilepticus seizures (Foresti *et al.*, 2009).

CCL2 has been identified as a systemic immune-related factor that critically contributes to the susceptibility of the aging brain to cognitive impairments, as systemic CCL2 in plasma has been correlated with reduced neurogenesis in the dentate gyrus in aged mice and deficits in synaptic plasticity (Villeda *et al.*, 2011). Heterochronic parabiosis experiments in mice show that blood-borne immune-related factors are contributing to

cognitive impairments, with contextual fear conditioning and hippocampal-dependent spatial learning and memory reduced when a young animal is exposed to an old systemic environment (Villeda *et al.*, 2011). Furthermore, human Alzheimer's disease studies have shown that higher levels of CCL2 promote a faster rate of cognitive decline (Westin *et al.*, 2012), and elevated CCL2 expression levels are related to lower memory factor scores in an assessment of episodic and visual memory (Bettcher *et al.*, 2016).

1.2.7 DARC

The Duffy antigen receptor for chemokines (DARC) is a glycosylated membrane protein located on the surface of erythrocytes and is expressed by the endothelial cells of the spleen, lung, thyroid, kidney and brain (Hadley and Peiper, 1997; Lee *et al.*, 2003), and binds to a number of pro-inflammatory cytokines of both the C-C family and C-X-C family (Gardner *et al.*, 2004). DARC (also referred to as ACKR1) is an atypical receptor belonging to the ACKR sub-family. Atypical receptors share the seven-transmembrane domain topology of conventional, typical, chemokine receptors, however the binding of a ligand to atypical receptors does not lead to G-protein recruitment. DARC is missing an Asp-Arg-Tyr consensus motif in its second cytoplasmic loop, and as a result, is incapable of transmitting intracellular signals when bound to chemokines and therefore does not directly act to translocate effector cells to sites of inflammation like traditional chemokine receptors (Pruenster *et al.*, 2009; Hadley and Peiper, 1997). The biological functions of DARC are different depending on its cellular expression. Erythrocyte DARC participates in physiological scavenging of chemokines and mediates chemokine concentration in the blood (Darbonne *et al.*, 1991; Dawson *et al.*, 2000). Unlike other atypical chemokine receptors, no degradation of chemokines occurs after their internalisation by DARC, but instead the receptor acts to remove surplus plasma chemokines that could mediate extensive and harmful inflammation (Mangalmurti *et al.*, 2009; Ransohoff, 2009). For example, DARC knockout animals receiving systemic lipopolysaccharide (LPS) injection responded with a marked increase in neutrophil infiltrate in the lungs and livers compared to wild-type controls (Dawson *et al.*, 2000), which suggests the receptor plays a role in chemokine homeostasis.

Endothelial DARC plays a subtle role in mediating inflammation as it internalises and transcytoses chemokines from their sites of synthesis to the luminal side of the endothelium, where the chemokines become embolised and contribute to leukocyte

extravasation to tissues (Pruenster *et al.*, 2009). Neutrophil and monocyte migration towards cognate chemokines is enhanced in monolayers expressing DARC (Lee *et al.*, 2003) and chemokine injections into mice over-expressing endothelial DARC have significantly greater leukocyte recruitment (Pruenster *et al.*, 2009). These experiments highlight that endothelial DARC acts to mediate internalisation and cellular transport of chemokines and prevents the escape of soluble tissue-derived chemokines into circulation and enhances firm adhesion of leukocytes (Zarbock *et al.*, 2010). Inflammation can further up-regulate DARC expression in veins and post-capillary venules and induce DARC to appear in vascular segments that are usually devoid of the receptor (Miller *et al.*, 1975; Segerer *et al.*, 2000; Patterson *et al.*, 2002; Geleff *et al.*, 2010).

CCL2 binds to DARC with high affinity (Hansell, Hurson and Nibbs, 2011), although the biological purpose of this function is not clear. DARC knockout mice have reported one-third lower basal CCL2 plasma levels compared to wild-type controls (Fukuma *et al.*, 2003), and humans lacking the gene encoding DARC had significantly lower basal CCL2 levels than DARC-positive humans (Jilma-Stohlawetz *et al.*, 2001). DARC has also been shown to have a role in shuttling inflammatory chemokines across the blood brain barrier (BBB) in *in vitro* models. DARC shuttles CCL2 and CCL5 across the BBB from the basolateral to the luminal side of mouse brain endothelial cells, and this transport was more efficient and significantly increased in TNFα stimulated brain endothelial cells in wild-type mice compared to mice lacking the DARC receptor (Minten *et al.*, 2014). These data suggest that DARC has a pro-inflammatory function in brain endothelial cells by shuttling chemokines across the BBB. DARC's immunoreactivity has also been observed at high levels in cerebellar Purkinje cells (Horuk *et al.*, 1996), and throughout the cortex in post-mortem brain tissue (Minten *et al.*, 2014). Single cell RNA sequencing data highlights that DARC is present in high abundance on non-myeloid brain cells (Schaum *et al.*, 2018), although its function and specific expression in neuronal subtypes in currently unknown.

1.2.7.1 DARC homozygous knockout mice

DARC knockout mice have been used to explore DARC function *in vitro* and *in vivo* (Benson *et al.*, 2018; Dawson *et al.*, 2000; Edderkaoui *et al.*, 2018). Using homozygous knockout mice, in which endothelial and erythrocyte DARC is fully eliminated, provides a good mechanism for exploring DARC-related functions.

In this thesis, a homozygous DARC knockout mouse line ($DARC^{-/-}$) was used to investigate the role of DARC in aging and hippocampal excitability. DARC deficiency in this mouse line was generated using gene targeting by Dawson and group. A 9-kilobase (kb) *Eco*RI fragment containing the DARC locus was cloned from an isogenic mouse genomic library. This fragment was used to generate the targeting construct shown in figure 1.8, which contained 4.3-kb and 1.2-kb arms of homology in the 5' and 3' regions, and a *neo* gene in opposite transcriptional orientation that replaced 90 base pairs (bp) of the DARC open reading frame. Embryonic stem cell lines that were correctly targeted were injected into C57Bl/6J blastocysts and subsequently implanted into CDI host pseudopregnant females to obtain 10 chimeric pups. Three chimer pups were able to transmit the DARC mutation through the germline when mated with C57BL/6J mice, resulting in F1 heterozygotes ($DARC^{+/-}$). F1 heterozygotes were intercrossed to generate F2 homozygous DARC-deficient mice, $DARC^{-/-}$ (Dawson *et al.*, 2000).

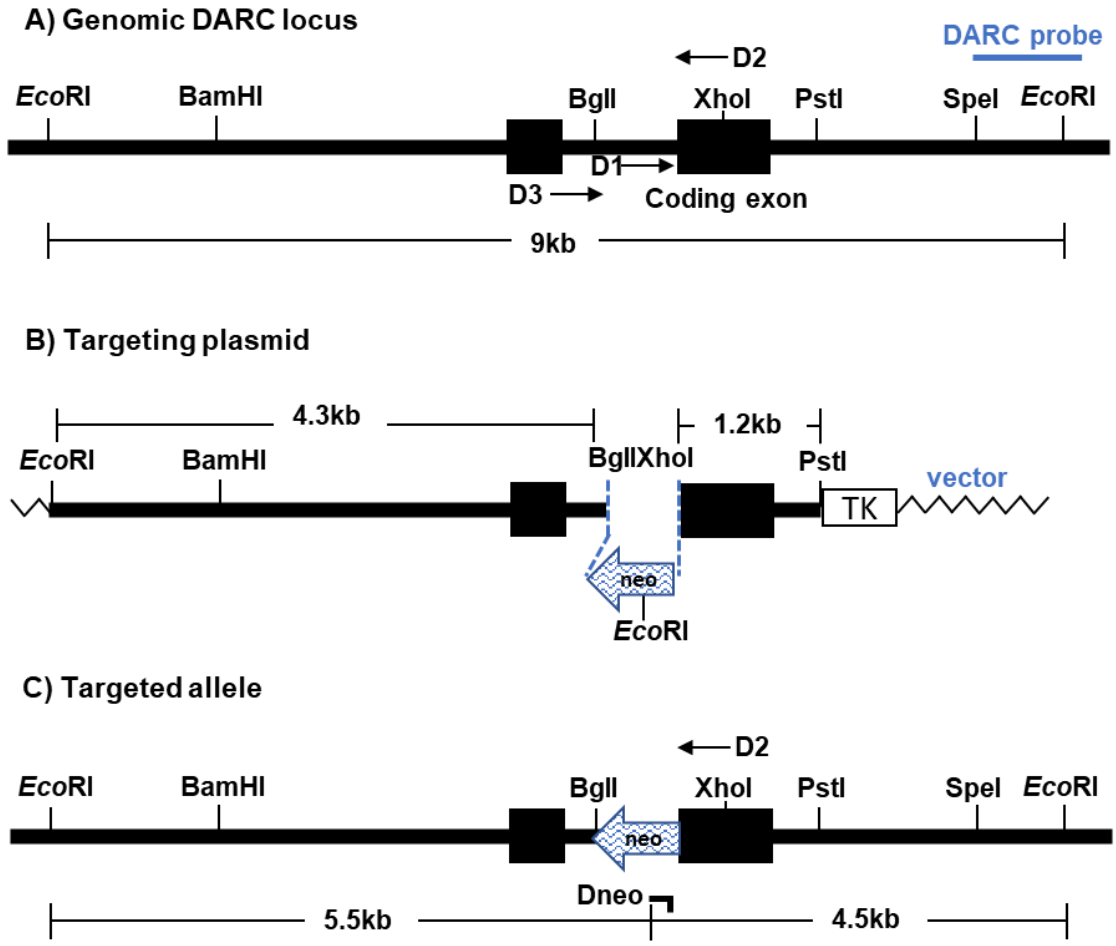


Figure 1.8 Gene targeting of the mouse DARC gene. A) Endogenous DARC gene locus showing exon I and exon II in a 9kb *Eco*RI fragment. A 3' 1-kb SpeI-*Eco*RI located outside the targeting construct was used by Dawson and group as a probe for Southern blot analysis. Primers D1 and D2 were used for PCR identification of the wild-type DARC gene, and primers D2 and D3 were used to detect DARC cDNA by RT-PCR analysis. B) The targeting plasmid, a 1.1kb neo gene, was inserted to replace the BglI-XhoI fragment, which eliminates a part of the intron and 90bp of exon II. A 5' 4.3kb *Eco*RI-BglI fragment was used as the 5' arm of homology and a 3' 1.2kb XhoI-PstI fragment containing most of the coding exon was used for the 3' homology. A TK gene was inserted for negative selection of embryonic stem cells. The OSDUPDEL vector was used to create the targeting construct. C) The correctly targeted allele - this can be detected by PCR analysis using primers D2 and Dneo^{-}. Figure adapted from Dawson et al., 2000.

1.3 Aims and Objectives

It is well described that hippocampal-dependent function declines in aged individuals which can lead to the onset of neurodegeneration. The dysregulation of innate immune responses in the peripheral and central nervous system in aging is thought to underlie the pathophysiological events leading to reductions in cognition and potential

neurodegenerative onset. Most studies of aging examine young age cohorts with elderly, but more recent evidence suggests that changes in hippocampal function occur at a younger age, as alterations in hippocampal-dependent behaviour, neural circuitry and upregulation of pro-inflammatory mediators have been observed in mid-aged individuals. There is, however, minimal investigation into whether similar changes are observed in early aging, and whether hippocampal function changes progressively with aging.

DARC is a non-signalling receptor that acts to mediate pro-inflammatory chemokine concentration in the blood. It has been shown to regulate the activity of the proinflammatory chemokine CCL2, which has been directly associated with age-related pathology of neurodegenerative disorders. DARC is also present in neurons, yet there has been no investigation into DARC function brain and whether it also mediates immune function in the cerebral environment.

In this thesis, I aim to investigate for changes in hippocampal activity during early aging and evaluate the role of the chemokine CCL2 and the chemokine receptor DARC in early age-dependent changes.

The main objectives are:

- Using *in vitro* electrophysiology, identify whether hippocampal activity along the perforant pathway is affected in early aging using young (9-15 weeks old) and adult (25-35 weeks old) wild-type mice.
- Investigate whether the pro-inflammatory chemokine CCL2 affects hippocampal function and microglia proliferation in early aging.
- Examine the role of DARC in hippocampal neuronal activity using *in vitro* electrophysiology and DARC homozygous knock out mice.
- Investigate whether DARC mediates CCL2 function in the hippocampus.

Chapter 2 – Materials and Methods

2.1 Materials

2.1.1 List of drugs and chemicals used

The following drugs were used in experiments described in this thesis:

Chemical	Supplier
Calcium Chloride	Sigma-Aldrich Ltd., Dorset, UK
Dimethyl sulfoxide (DMSO) 99.5%	Sigma-Aldrich Ltd., Dorset, UK
Glucose	Fischer Scientific Limited., Leicestershire,
Magnesium sulphate	Sigma-Aldrich Ltd., Dorset, UK
Normal Goat Serum	Sigma-Aldrich Ltd., Dorset, UK
Isoflurane (Vetflurane)	Virbac, UK
Paraformaldehyde	Fischer Scientific Limited., Leicestershire,
Phosphate buffered saline	Gibco., UK
Potassium acetate	Sigma-Aldrich Ltd., Dorset, UK
Potassium chloride	Fischer Scientific Limited., Leicestershire,
Sodium bicarbonate	VWR International Ltd., Leicestershire, UK
Sodium chloride	VWR International Ltd., Leicestershire, UK
Sodium dihydrogen phosphate	VWR International Ltd., Leicestershire, UK
Sucrose	Fischer Scientific Limited., Leicestershire,
Triton X-100	Sigma-Aldrich, UK
Tween-20	Sigma-Aldrich, UK
ABTS Solution	Sigma-Aldrich, UK

Drug	Supplier	Stock Conc.	Working Conc.	Vehicle
Kainic acid	Tocris, Bristol, UK	1mM	200nM	H_2O
Ketamine (Ketavet)	Zoetis Ltd., UK	100mg/ml	100mg/kg^{-1}	N/A
Recombinant Murine CCL5	PeproTech, New Jersey, USA	10μg/ml (1.3uM)	10ng/ml (1.3nM)	H_2O
Recombinant Rat CCL2	Cell Guidance Systems Ltd., Cambridge, UK	100μg/ml (7uM)	0.1μg/ml (7nM)	H_2O
RS504393	Tocris, Bristol, UK	10nM	1μM	DMSO
SB328437	Tocris, Bristol, UK	100nM	8nM	DMSO
Rompun (Xylazine)	Bayer, York, UK	20mg/ml	10mg/kg^{-1}	N/A

Stock solutions were obtained by diluting stock solids with double-distilled H_2O or dimethyl sulfoxide (DMSO). DMSO was used at a final concentration no greater than 0.05% (v/v) when added to circulating ACSF. All drug stock solutions were administered to slices via bath application to the circulating ACSF.

2.1.2 ACSF formulations

Normal artificial cerebral spinal fluid (nACSF) was comprised of 126mM NaCl, 3mM KCL, 1.25mM NaH_2PO_4, 1mM $MgSO_4$, 1.2mM $CaCL_2$, 24mM $NaHCO_3$ and 10mM glucose. Sucrose artificial cerebral spinal fluid (sACSF) solution used for intracardial perfusion of animals and brain cutting solution was composed of 252mM sucrose, 3mM KCL, 1.25mM NaH_2PO_4, 24mM $NaHCO_3$, 2mM $MgSO_4$, 2mM $CaCL_2$, and 10mM glucose.

nACSF contained a reduced concentration of $MgSO_4$ and $CaCl_2$ ions than sACSF to increase the level of intrinsic excitability of slices. Extracellular Mg^{2+} blocks NMDA receptors, and reducing extracellular Mg^{2+} below the physiological level greatly potentiates the NMDA response (Nowak *et al.*, 1984). In addition, lowering extracellular Ca^{2+} concentration increases intrinsic bursting of fast, rhythmically bursting cells in the

hippocampus (Su *et al.*, 2001). sACSF, which has an equimolar replacement of sodium chloride (NaCl) and sucrose, has been shown to improve neuronal preservation by reducing the passive influx of sodium ions and subsequent water entry and cell swelling during slice cutting (Aghajanian and Rasmussen, 1989).

2.2 Animal provision

All procedures were conducted in accordance with the regulations detailed in the UK Animals (Scientific Procedures) Act, 1986. All appropriate project and personal licences were granted by the UK Home Office.

Experiments were carried out on wild type C57Bl/6J and DARC$^{-/-}$ (DARCko, ACKR1$^{-/-}$) male mice from two age cohorts, 9-15 weeks, and 25-35 weeks old. C57Bl/6J mice were obtained from Jackson Laboratories and were used throughout experimentation as a control cohort. The original DARC$^{-/-}$ mouse breeders were obtained from the Dawson laboratory, and further offspring were bred in house within the Biological Services Facility at the University of York. The DARC$^{-/-}$ mice were originally generated as described previously (Dawson *et al.*, 2000). Homozygous DARC knockout males were bred with homozygous DARC knockout females to generate offspring of the same genotype. As male mice were only used for experimentation, female littermates were terminated; a small number of females were kept every 2-3 generations for breeding purposes. Family lineages were closely monitored to reduce the incidence of inbreeding and no littermate or parent-offspring breeding was conducted. To mitigate the impact of genetic drift, DARC$^{-/-}$ males were backcrossed with C57BL/6J females on two occasions during the experimentation period. On each occasion, 3 lots of breeding trios were set up, composed of one DARC$^{-/-}$ male and two C57Bl/6J females. DARC males were from different family lineages. The resultant offspring, all DARC heterozygous pups (DARC$^{+/-}$), were genotyped to confirm correct genetic background. Once of breeding age, 3 breeding trios comprised of all DARC$^{+/-}$ were created (males and female breeders within a trio were taken from different litters). The resultant offspring, with possible genetic backgrounds of either wild type, DARC heterozygous knockout or DARC homozygous knockout, were genotyped. Once identified, homozygous DARC knockout males were bred with DARC homozygous knockout females to produce all DARC homozygous knockout pups.

Animals were group housed with up to 5 individuals per cage, on a 12-hour light-dark cycle, with *ad libitum* supply of food and water.

Genotype testing was assessed at the point of weaning via DNA extraction and PCR protocol.

2.3 Anaesthesia and animal procedures

Animals were introduced to a five litre bell jar and exposed to approximately 3ml of inhalant anaesthetic isoflurane (Virbac, York, UK), administered at a concentration sufficient to abolish righting reflex. Once righting reflex was tested, intramuscular injection of ketamine (100mg/kg^{-1}, Zoetis Ltd, London, UK) and xylazine (20mg/kg^{-1}, Bayer, York, UK) was administered into the gluteal area of the hind leg. Pain reflexes were tested by pedal withdrawal and blink reflexes, and once confirmed, the thoracic cavity and rib cage of the animal was opened to expose the heart. A catheter was inserted into the left ventricle of the heart and an incision made into the right atrium. Animals were then intracardially perfused with approximately 25ml of ice-cold, oxygenated sucrose artificial cerebral spinal fluid (sACSF) using a peristaltic pump (Watson-Marlow, UK) at a rate of 1ml/s. Following intracardial perfusion, the spinal cord was severed, and an incision was made along the head to expose the skull. The skull was cut along the midline from a caudal to rostral direction and the skull removed to expose the brain. The brain was removed and immediately immersed in a Petri dish of ice-cold, oxygenated sACSF, before the cerebellum and olfactory bulbs were removed using a scalpel and discarded.

2.4 Preparation of hippocampal slices

The brain was glued to the chuck of a Leica VT100 S vibratome (Leica Microsystems, Nussloch GmbH, Germany) on its horizontal surface, before securing the chuck into the vibratome cutting chamber, filled with ice-cold, oxygenated sACSF. Intracardial perfusion and immersion with sACSF is a widely adopted method for preparing acute brain slices, based on the premise that passive sodium influx and subsequent water entry and cell swelling during slice cutting leads to a poor survival of neurons.

Horizontal sections were cut at a thickness of 450µm, a measurement thin enough to allow adequate oxygenation of slices and thick enough to ensure that local networks are

preserved and sufficiently intact. Horizontal sections were used for perforant pathway investigation, as medial and lateral perforant pathway fibres remain intact in this orientation (Van Hoeymissen *et al.*, 2020). Slices were transferred to a petri-dish filled with ice-cold, oxygenated ACSF, and slices of the midbrain containing dorsal entorhinal cortex and hippocampal structures were selected (an average of 3 suitable slices were obtained from each brain). Slices were trimmed with a scalpel so that only the hippocampus and connecting entorhinal cortex remained (Fig 2.1). 2 hippocampal sections were obtained from each brain slice.

2.5 Maintenance of hippocampal slices

Hippocampal slices were transferred to an interface recording chamber and placed on double-thick lens cleaning tissue and maintained in an oxygenated, humidified environment (caroben gas, 95% O_2 / 5% CO_2) at an interface with continuously circulating oxygenated normal ACSF (50ml), pumped using a peristaltic pump at a rate of 2ml/min. Slices were left for 30 minutes before the temperature of the circulating nACSF was gradually raised to 32ºC using a flow heater (Grant Instruments Ltd., Cambridge, UK) and slices were left to equilibrate to the recording chamber for a further 1 hour prior to control recordings. Spare slices were stored in a holding chamber in a humidified environment at an interface with oxygenated nACSF until their use and transfer to the recording chamber.

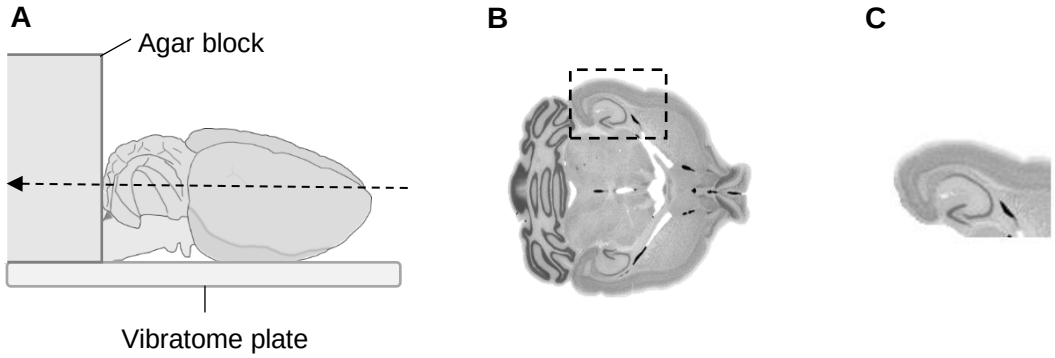


Figure 2.1 Schematic representation of hippocampal slice generation. (**A**) Mouse brains were removed and secured onto a vibratome plate against an agar block for cutting support. 450µm horizontal sections were cut using a vibratome in the direction indicated by the dashed arrow. This generated slice indicated in (**B**). Slices were trimmed using a scalpel so that only the hippocampus and connecting entorhinal cortex remained. (**C**) Representation of a trimmed hippocampal section that would be used for *in vitro* electrophysiology.

2.6 Electrophysiology recording techniques

Extracellular field potentials were detected using micropipettes pulled from thin walled borosilicate glass capillaries (1.2mm O.D., 0.94mm, I.D., Harvard Apparatus, Kent, UK) using a P-1000 flaming/brown horizontal micropipette puller (Shutter Instruments Co., California, USA). Pipettes were filled with normal ACSF of the same ionic composition as that in which the slices are maintained before being placed in pipette holders containing silver wire inner filament to form a conducting bridge between the nACSF and amplifier electronics. Pipette holders were attached to micromanipulator head stages (HS-9A Axon Instruments) to allow fine movements and precise electrode placement. Extracellular pipettes had a resistance between 2-5MΩ. The electrical signal from each brain slice was conducted through the micropipette nACSF solution to the silver wire, amplified (Axoclamp 900A, Axon Instruments, California, USA) and digitised (ITC018 A/D converter, HEKA Instruments) at a sampling frequency of 2000Hz and band-pass filtered between 0.1 and 300Hz. All electrical noise at 50Hz was filtered from all signals using Humbugs (Quest Scientific, Vancouver, Canada).

2.6.1 Stimulus response recordings

Once the slices had been allowed to equilibrate in the interface chamber for 1 hour, extracellular electrodes were placed in the supra-pyramidal and infra-pyramidal subregions of the dentate gyrus as represented in figure 2.2 A, which was facilitated using a stereo microscope and micromanipulators to aid electrode placement. To mimic perforant pathway activity *in vitro,* a stimulating electrode connected to a DS2A isolated stimulator box (Digitimer Ltd.,) was placed in layer II of the entorhinal cortex, and an initial stimulation of 30V was used to check perforant pathway connection between the entorhinal cortex and dentate gyrus. If field excitatory post synaptic potentials (fEPSPs) were detected from the dentate gyrus using Axograph software (version 1.7.6), control recordings were taken by connecting the stimulator box to a Master 8 pulse stimulator (A.M.P.I, Jerusalem, Israel), which initiated 10 voltage stimulations (200ms duration) at 1 second intervals. The subsequently generated fEPSPs were recorded simultaneously from the supra-pyramidal and infra-pyramidal subregions of the dentate gyrus, with the average of 10 input stimulations recorded. Input voltage began at 5V and increased in regular intervals up to a maximum stimulation of 100V, to generate a stimulus response

curve. The rising slope (Fig 2.2 B) of the fEPSP for each input voltage was measured using a toolbar on the Axograph software.

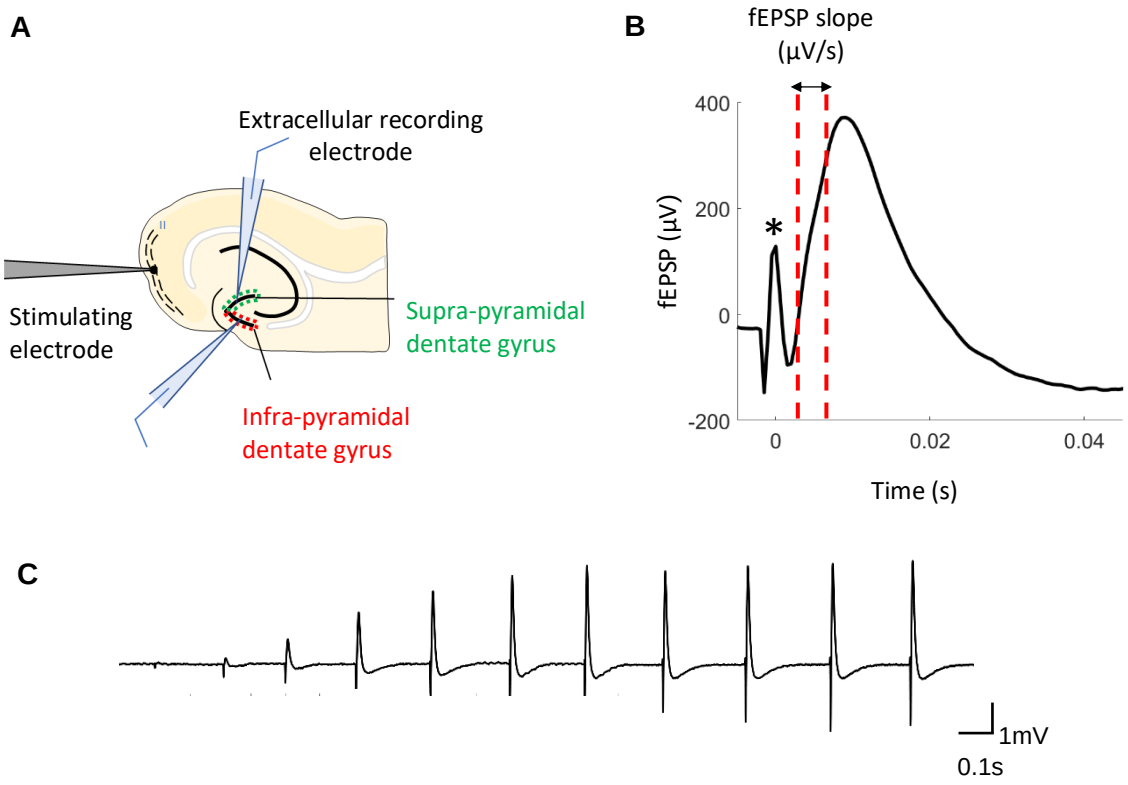


Figure 2.2 Schematic diagram of electrode placement and fEPSP generation. (**A**) Schematic diagram of a hippocampal slice highlighting electrode placement and the supra-pyramidal (green) and infra-pyramidal (red) subregions of the dentate gyrus. (**B**) Example of a field excitatory post synaptic potential (fEPSP) trace recorded from the supra-pyramidal dentate gyrus from a wild-type mouse, generated by 30V stimulation of layer II of the entorhinal cortex. The red dashes indicate the region of the fEPSP slope measured. * indicates the stimulus artefact. (**C**) Example of fEPSPs generated from entorhinal cortex layer II stimulation at each intensity (5V-100V). fEPSPs represent the average response of 10 electrical stimulations measured from the supra-pyramidal dentate gyrus in a wild-type mouse.

2.6.1.1 Investigating the effects of DMSO on perforant pathway activity

Some pharmacological compounds used for experimentation within this thesis (RS504393 and SB328437) were reconstituted in the polar aprotic solvent dimethyl sulfoxide (DMSO). Evidence indicates that DMSO added to nACSF at a concentration of 0.05% (v/v), alters intrinsic excitability properties of hippocampal CA1 pyramidal neurons (Tamagnini *et al.*, 2014). The concentration of DMSO in the reconstituted compounds was calculated to be approximately 0.01% (v/v) once added to 50ml circulating nACSF. This experiment aimed to investigate whether a concentration of 0.01% (v/v) affected neuronal excitability along the perforant pathway.

A stimulus response curve was measured from the suprapyramidal dentate gyrus in hippocampal slices bathed in circulating nACSF as described in 2.6.1, which acted as an internal control. DMSO was then directly added to circulating ACSF for a bath concentration of 0.01% (v/v). Hippocampal slices were incubated in DMSO for 1 hour before a second stimulus response curve was generated. The rising slope of the EPSPs that were generated from perforant pathway stimulation, were measured in the control hippocampal slices and from the same slices that had been incubated in DMSO – the differences in fEPSP slope measured before and after DMSO addition were calculated.

2.6.1.2 Investigating the effects of CCL2 on perforant pathway activity

To investigate the effects of CCL2 on perforant pathway activity, a stimulus response curve was generated as described in 2.6.1. As hippocampal slices were bathed in only circulating nACAF, this was used as a control sample. After the control stimulation curve was generated, 7µM of recombinant rat CCL2 was added via bath application to circulating nACSF (for a bath concentration of 7nM), and slices were left to incubate in circulating CCL2 for 1 hour. This concentration was selected as previous electrophysiology studies have demonstrated that acute concentrations of 2.3 nM and 10nM CCL2 increase neuronal excitability (Gruol *et al.*, 2014; Guyon *et al.*, 2009; Zhou *et al.*, 2011; Belkouch *et al.*, 2011). In addition, 7nM CCL2 is the recommended working concentration provided by the manufacturer (Cell Guidance Systems) based on its IC50 value. Therefore, 7nm CCL2 was used for experimentation throughout this thesis. After the 1 hour incubation with CCL2, a second stimulus response curve was generated, and the rising lope of fEPSPs were measured to compare how CCL2 incubation affects fEPSPs in the dentate gyrus upon perforant pathway stimulation.

2.6.1.3 Investigating whether CCL2 activity is mediated through CCR2 binding

CCL2 exerts its proinflammatory functions by binding to its cognate G protein-coupled receptor CCR2 (Deshmane *et al.*, 2009; Pinke *et al.*, 2013). To investigate if CCL2 was exerting its effects in hippocampal neurons through binding to CCR2, a CCR2 receptor antagonist, RS504393, was used to block most CCR2 function. This experiment was performed in a similar manner as described in 2.6.1.2, however slices were pre-bathed in 1 µM RS504393 (made up in DMSO) to block CCR2 function. DMSO has been previously described to affect hippocampal slice activity, therefore, to eliminate the possibility of DMSO directly affecting hippocampal activity instead of/in addition to the

pharmacological compound itself, slices were pre-bathed in RS504393 (in DMSO) for 1 hour before a "control" recording was taken. This control recording in this thesis is described as "+ RS504393" herein. CCL2 was then added to circulating nACSF for a bath concentration of 7 nM. Another stimulus response curve was measured from slices after 1 hour of CCL2 incubation. A schematic of the experimental paradigm and sequence of pharmacological compounds is represented in figure 2.3. fEPSP slopes were measured from "+ RS504393" stimulation curves and post CCL2 stimulation curves in order to investigate the effects of CCL2 addition on hippocampal slices bathed in a CCR2 receptor antagonist.

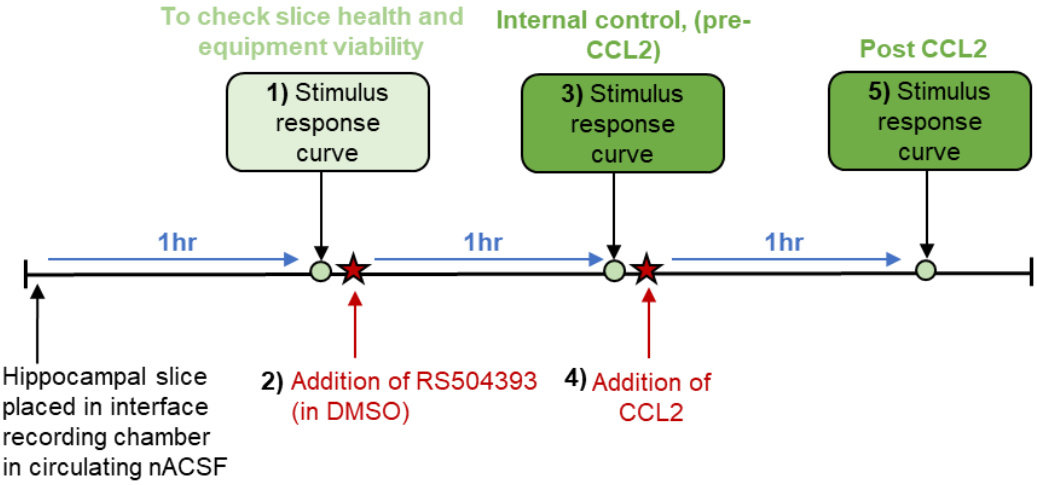


Figure 2.3 Schematic diagram representing experimental paradigm and sequence of pharmacological compound addition. Hippocampal slices were placed in the interface recording chamber in circulating nACSF and left to acclimatise to environmental conditions for 1 hour. 1) A stimulus response curve was taken from slices bathed in ACSF to ensure slice health and equipment viability. 2) The CCR2 receptor antagonist RS504393 (reconstituted in DMSO) was added to circulating nACSF for a bath concentration of 1 µM. Slices were incubated in RS504393 for 1 hour. 3) A stimulus response curve was then recorded from hippocampal slices that had been incubated in RS504393. This stimulus response curve was used as a "pre-CCL2" control sample – any changes in hippocampal excitability caused by DMSO would be accounted for in this control sample. 4) CCL2 was then added directly to circulating nACSF for a bath concentration of 7 nM and slices were incubated for 1 hour. 5) A final stimulus response curve was measured from hippocampal slices.

2.6.1.4 Investigating the effects of CCL5 on perforant pathway activity

To investigate the effects of CCL5 on perforant pathway activity, a stimulus response curve was first generated in hippocampal slices bathed in circulating nACSF as an internal control, as described in 2.6.1. After the control stimulus response curve was generated, 1.3 µM of recombinant murine CCL5 was added via bath application to circulating nACSF (for a bath concentration of 1.3 nM). 1.3 nM (10ng/ml) was selected as a concentration as this was the recommended working concentration from the manufacturer (PeproTech, Germany) based on CCL5's ability to chemoattract human activated T-cells. Hippocampal slices were incubated in CCL5 for 1 hour before a second stimulus response curve was generated to investigate how CCL5 affects fEPSP responses.

2.6.2 Gamma oscillation recordings

Hippocampal slices were left to equilibrate in the interface chamber for thirty minutes. An extracellular electrode was then placed in entorhinal cortex layer II and another in the CA3 (Fig 2.4 A). Electrode positioning was facilitated using a stereo microscope and micromanipulators. A 1-minute control recording was taken from both subregions before Kainic acid (200 nM) was then applied to the circulating nACSF. A 200 nM bath concentration was sufficient to generate gamma oscillations in both the entorhinal cortex and CA3, as discovered by kainic acid concentration curve experiments represented in figure 2.4 B. 1-minute recordings were taken every 10 minutes to measure for the presence of gamma oscillations. The presence of gamma oscillations were measured using the software Axograph 4.6/X (Axon Instruments, USA), which converted raw 1 minute extracellular recordings into power spectra (specific details of this transformation are described in 2.6.3). If hippocampal slices were oscillating at the gamma frequency, a peak was observed in the power spectrum in the gamma frequency range (25-100Hz), as demonstrated in figure 2.5. Slices were considered to have generated gamma oscillations if the peak power was between 25-100Hz and was above $10\mu V^2$. Oscillations were considered to be stable once the peak power of the oscillation did not change by more than 10% in a 30-minute window. Gamma experimental recordings were only taken once gamma had fully stabilised in both entorhinal cortex and CA3 regions. On average, stabilisation of both subregions took between 1.5-3 hours. Once gamma had stabilised, a 1-minute recording was taken from both subregions and saved.

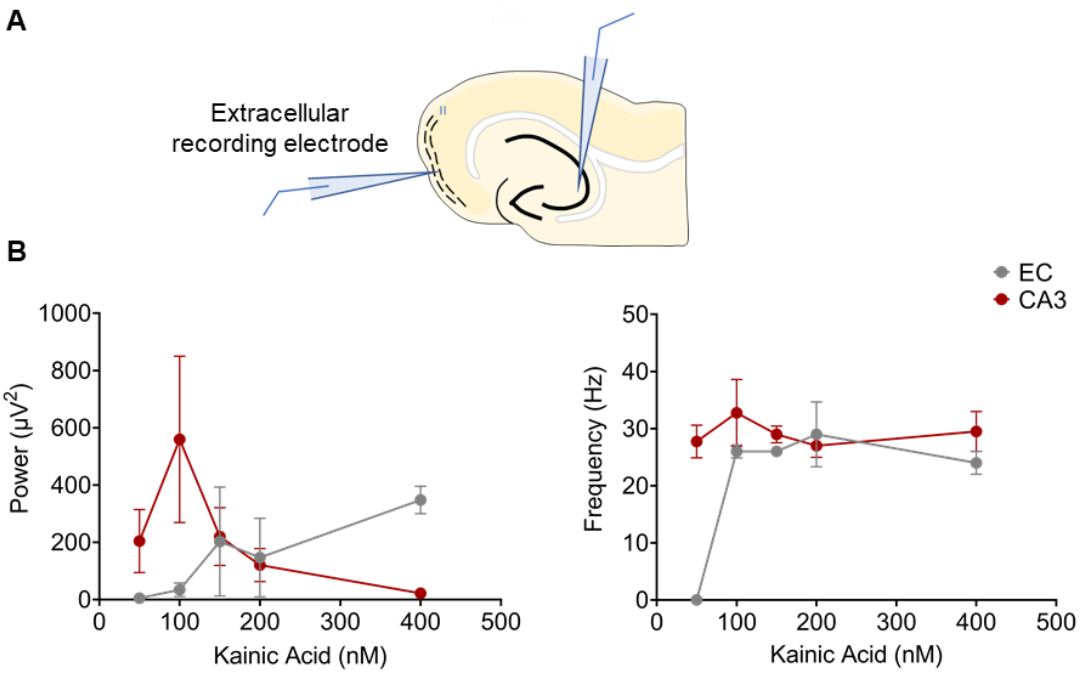


Figure 2.4 200nM of kainic acid generates robust gamma oscillations in the entorhinal cortex and CA3 regions. Hippocampal slices were prepared from 9-15 week old wild-type mice. 1mM kainic acid was added to circulating artificial cerebral spinal fluid in cumulative amounts to achieve 50nM, 100nM, 150nM, 200nM and 400nM bath concertation. Oscillations were recorded at each kainic acid concentration from the entorhinal cortex and CA3 until the oscillations had stabilised. Gamma oscillation power and frequency were plotted at each kainic acid concentration to determine a concentration able to induce robust gamma in both subregions. (**A**) Representation of entorhinal cortex and CA3 electrode placement. (**B**) The power and frequency of gamma oscillations were measured from the entorhinal cortex and CA3, after addition of 50 nM – 400 nM kainic acid. (**B**) Bars represent mean ± SEM, N=4 mice.

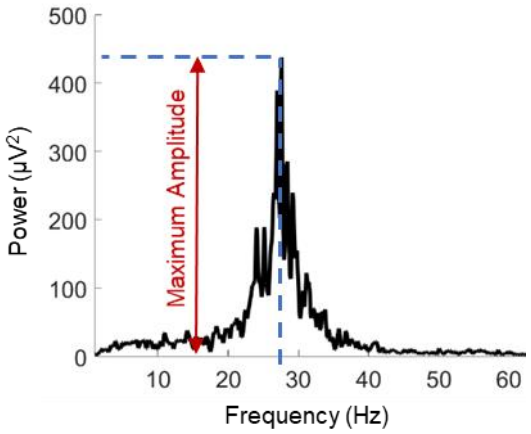


Figure 2.5 Example of power spectra generated from 1 minute epochs of raw extracellular gamma oscillations measured from the hippocampus. Power spectra were generated using the Fast Fourier Transform algorithm in the Axograph 4.6/X (Axon Instruments USA) software. The modal peak power and frequency of oscillations were measured, which corresponded to the largest peak amplitude in the spectra, and the frequency domain at which this largest peak was situated (highlighted by the blue dashed line). If the largest peak amplitude was between 25-100Hz and greater than 10μV^2, hippocampal slices were considered to be oscillating at the gamma frequency.

2.6.3 Analysis of network oscillations

Analysis of raw data was conducted using the Axograph 4.6/X (Axon Instruments, USA) and Matlab 2018b (The MathsWorks Inc., Massachusetts, USA) software packages. Subsequent statistical analysis was conducted using GraphPad Prism 9 (San Diego, California, USA).

The properties of the gamma oscillatory activity detected by extracellular, local field potential recordings were quantified according to several parameters. These included the modal peak frequency and peak power (amplitude) of the oscillation, and the phase locking value of oscillations between the entorhinal cortex and CA3.

Power spectra were created from 60 second epochs of raw extracellular gamma oscillation recordings using a Fast Fourier Transform (FFT) algorithm. The FFT converts the waveform of the oscillation from a time domain to a frequency domain by deconstructing the oscillation into its constituent sine wave components and calculates the power of each wave. This is expressed as a plot of the sum of the voltage squared at a given frequency ($\mu V^2/Hz$). The frequency and modal peak power of the oscillations were taken from the power spectra.

The phase locking relationship of the oscillatory activity between the entorhinal cortex and CA3 was assessed using Matlab software by linear cross-correlation analysis. Phase locking refers to a situation in which phases of two dependant generated oscillations are related linearly and are oscillating with the same frequency. Phase locking demonstrates greater synchrony between two regions. Interregional gamma synchrony has been proposed as a mechanism to precisely coordinate neuronal assemblies (Singer, 1993; Engel, Fries and Singer, 2001; Fries, 2009; Buzsáki and Wang, 2012) and synchronisation of gamma oscillations between the entorhinal cortex and hippocampus is associated with successful memory encoding in humans (Fell *et al.*, 2001) and macaques (Jutras, Fries and Buffalo, 2009). The 60 second epochs of gamma oscillation data recorded from the entorhinal cortex layer II and CA3 was analysed to determine the phase locking value of oscillations between the two regions by comparing the individual traces at multiple time delays. Phase locking was determined by comparing the amplitudes of the central wave peaks as they crossed the Y-axis at the zero time point. The oscillations were considered in phase if they reached their maximum

displacement at the same time point. Two oscillations with 100% phase locking were given a value of 1, and oscillations with no phase locking a value of 0.

2.6.4 Intracellular recordings

Sharp tip microelectrodes used for intracellular recordings were pulled from borosilicate glass capillaries (1.2mm O.D, 0.69mm I.D., Harvard Apparatus, Kent, UK), containing a silver wire inner filament and had a resistance in the range of 70-150MΩ and were filled with 2M potassium acetate solution. Intracellular signals were amplified, digitised and filtered using equipment described in section 2.6, however signals were recorded in direct current (DC) mode, digitised at 10KHz and low pass filtered at 2KHz. A stimulating electrode was first placed in the entorhinal cortex layer II and intracellular measurements were recorded from granule cells in the molecular layer of the dentate gyrus from the supra-pyramidal and infra-pyramidal subregions. Sharp tip electrodes were tracked through the 450µM slice using micromanipulators, with a -0.2na hyperpolarising holding current and tuning step (-0.2nA at 2Hz) applied. Piercing of granule cells was aided by using the capacitive buzz function of the amplifier, which resonated the electrode tip in the tissue helping to pierce the neuronal membrane. Once the membrane was penetrated, the holding current was hyperpolarised to help stabilise the cell and from a seal between the membrane and electrode. Approximately five minute recordings were taken of each cell measuring resting membrane potential, membrane resistance and excitatory postsynaptic potentials generated by stimulation of the entorhinal cortex layer II at 100% maximum stimulation and 50% maximum stimulation. 50% maximum stimulation was determined by first measuring the peak amplitude of the EPSP at maximum (100%) stimulation. The 50% maximum stimulation was subsequently the stimulation required to generate EPSPs with amplitudes 50% of the amplitudes recorded at maximum stimulation. Due to the low resting membrane potential of the granule neurons, an injection of positive current was unable to depolarise the membrane potential enough to generate inhibitory post synaptic potentials.

2.6.5 Analysis of Intracellular recordings

Intracellular recordings taken from the dentate gyrus granule cell layer were analysed using Axograph software. Resting membrane potential was measured by averaging the membrane voltage of 1 minute intracellular recordings, using a line tool to accurately determine the membrane potential. EPSPs that were generated via perforant pathway

stimulation of the entorhinal cortex layer II, and the amplitude of subsequent EPSPs generated were also measured using a line tool of the Axograph software (Fig 2.6 A).

To determine membrane resistance of granule neurons, the change in voltage output to changing current inputs were measured using the Axograph measuring tool as shown in figure 2.6 B. The resultant data was plotted as a voltage current curve, and membrane resistance was calculated using Ohms law (Resistance = Voltage / Current).

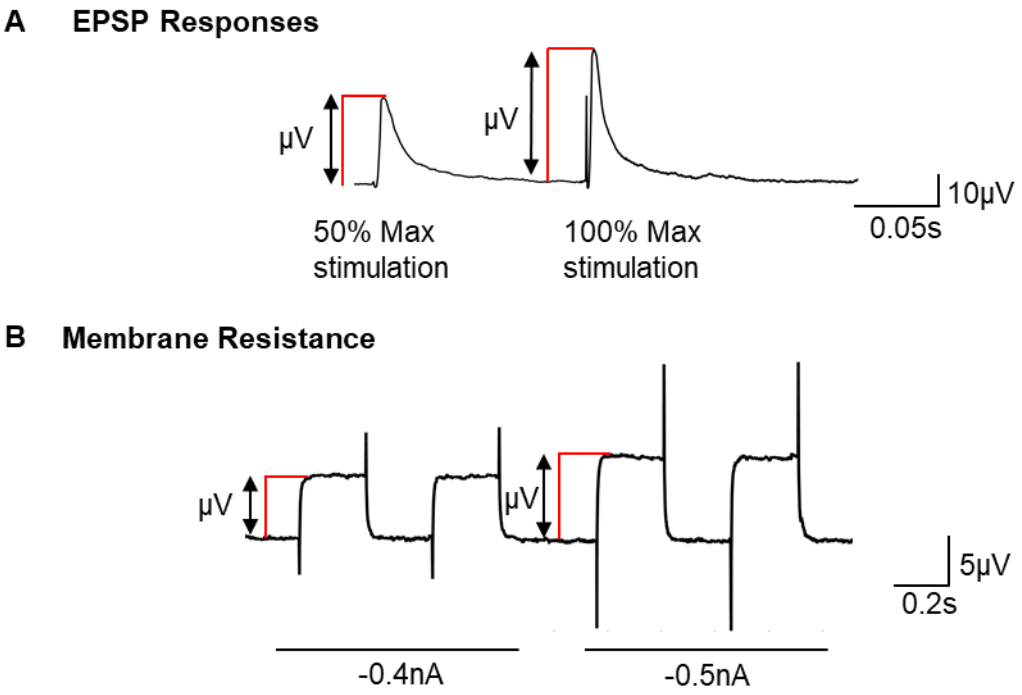


Figure 2.6 Intracellular EPSP and membrane resistance measurements of dentate gyrus granule neurons. (**A**) Examples of EPSPs generated from a supra-pyramidal dentate gyrus granule cell after 50% maximum and 100% maximum perforant pathway stimulation. Arrows indicate measurements of EPSP amplitudes. (**B**) Example of membrane voltage changes measured from a supra-pyramidal granule dentate gyrus granule neurone after -0.4nA and -0.5nA of input current was injected into the cell. Arrows indicate measurements of voltage change, which were plotted against input current to calculate membrane resistance using Ohm's law.

2.7 Rodent behavioural experiments

Mice were handled for 3 days prior to behavioural experiments to condition the animals to the tunnelling procedure and basic handling. Animals were acclimatised to the procedure room for 30 minutes before commencing the behavioural experiment and all trials were conducted under dim lighting to alleviate stress. All behavioural mazes and arenas were novel to the mice.

2.7.1 Y-maze spontaneous alternation test

The Y-maze spontaneous alternation test measures a rodent's willingness to explore new environments and is used as a measure of spatial working memory (Ukai, Shinkai and Kameyama, 1996). A Y maze is comprised of three arms that are 120º angles from each other, made from white high-density non-porous plastic (as displayed in figure 2.7). Spontaneous alternation is achieved when an animal enters a new arm rather than returning to one visited previously. As rodents typically prefer to investigate new environments, they usually show a tendency to enter a less recently visited arm (Hughes, 2004; Bak *et al.*, 2017).

Mice were placed into the Y-maze using a cardboard tube, always starting in the same arm, and facing the centre of the maze. The mice were left to explore the maze for 5 minutes and movements were recorded by a webcam situated above the maze. The inside of the Y-maze was cleaned with 70% ethanol and double-distilled water between trials and allowed to dry.

The behavioural experiment was scored by hand and percentage alternation was calculated using the equation: (no. of alternations) / (total no. of arm entries -2) x100, where an alternation represents a mouse entering each 3 arms of the maze consecutively.

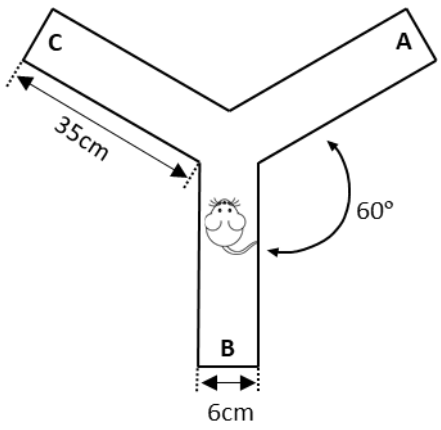


Figure 2.7 Schematic diagram of Y-maze used for spontaneous alternation testing. The maze is comprised of 3 arms, labelled A-C, 120º apart (width 6cm, length 35cm and height 20cm). The diagram of the mouse indicates the starting position of each rodent.

2.7.2 Open field

The open field test assess rodents general locomotor activity levels, anxiety and willingness to explore an environment (Denenberg, 1969). The behavioural experiment involves the use of an arena made from white high-density non-porous plastic (width 50cm, length 50cm, height 38cm), divided into an inner and an outer zone as represented in figure 2.8 A. A mouse was placed in the centre of the arena, transferred from its home cage using a cardboard tube, and its behaviour recorded for 10 minutes using an overhead camera. The arena was cleaned between trials using 70% ethanol and double-distilled water and allowed too fully dry.

2.7.2.1 Analysis of open field experiment

10 minute video clips were saved as an MP4 file and imported and analysed using Matlab 2018b (The MathsWorks Inc., Massachusetts, USA). An automated script used to measure a variety of locomotion parameters was obtained from (Zhang, Li and Han, 2020). The core of the program works by inverting the greyscale image of each video frame, converting it to a binary image based on a user-defined threshold, and measuring the position of the mouse in the binary image. The program tracks the trajectory of each mouse and calculates total distance travelled, time spent in the inner and outer zones, distribution of mouse area, thigmotaxis and a map of mouse trajectory for each 10 minute video recording. An example of thresholding and mitotracking of mouse trajectory is displayed in figure 2.8 B.

Mouse rearing behaviour, in which the animal stands on its hindlegs, is shown to be a measure of anxiety-related behaviour (Sturman, Germain and Bohacek, 2018). This parameter was measured by manually counting the number of rears each animal executed in the 10 minute video recording. Each video was scored twice, and a mean average calculated if different rearing scores were obtained for the repeated measurements.

Subsequent statistical analysis was conducted using GraphPad Prism 9 (San Diego, California, USA).

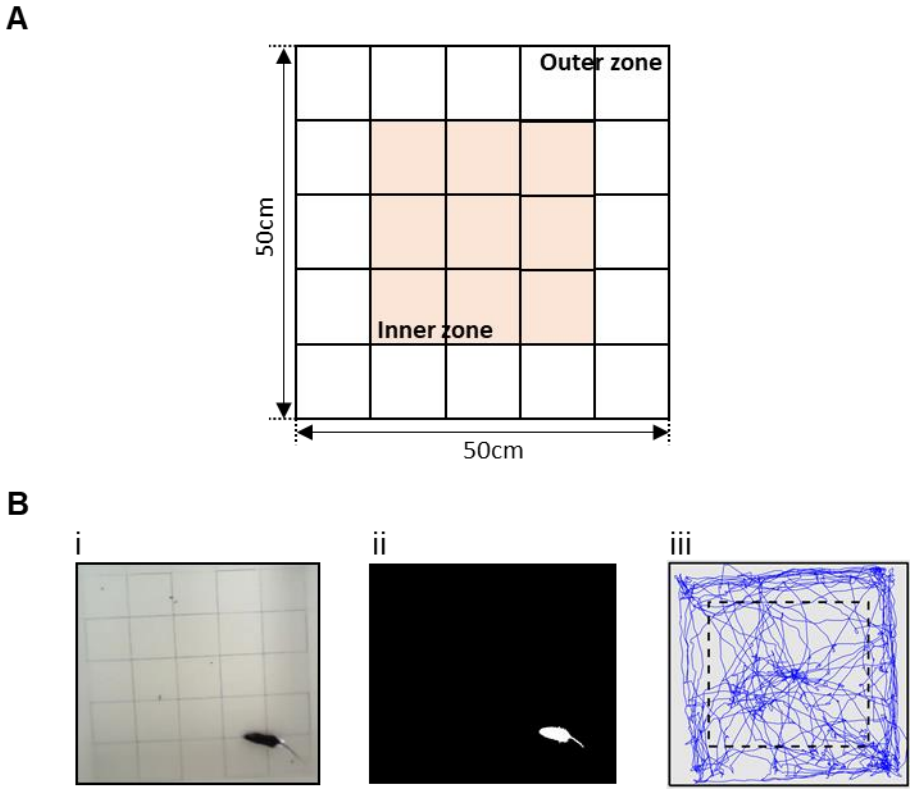


Figure 2.8 Open field arena behavioural analysis. (**A**) Schematic of an open field arena. A 50cm width x 50cm length x 38cm height arena made from white non-porous plastic was divided into an inner and outer zone. Rodents were placed in the centre of the inner zone as a starting point and movement trajectory was video recorded for 10 minutes. (**B**) Example of mitotracking software used for the open field behavioural experiment. i) Example image of a single video frame taken from a 10 minute recording of a wild-type mouse in the open field arena. ii) An example of thresholding video to mitotrack rodent movements. iii) Example trace of mouse movement trajectory over a 10 minute recording period. Dashed lines indicate the inner zone area.

2.8 Mouse genotyping

2.8.1 Preparation of genomic DNA

DARC knockout male mice were genotyped before experimentation to confirm that the DARC gene was not expressed. Ear notch samples were taken from weaned DARC knockout male mice at approximately 21 days old and genotyped using a polymerase chain reaction protocol. Preparation of genomic DNA was achieved by storing each ear notch in a 1.5ml Eppendorf tube and adding 600µl of tissue lysis buffer (25mM Tris pH 8.0, 10mM EDTA pH8.0, 1% SDS) and 3µl Proteinase K (200mg/ml, Promega), before leaving to lyse overnight at 55ºC. 200µl of protein precipitation solution (7.5M ammonium acetate) was added to each sample and vortexed thoroughly before centrifuging at

15,000 rpm for 3 minutes. The supernatant was decanted into a fresh tube containing 600µl isopropanol. The Eppendorf tubes were inverted approximately 50 times to mix before centrifuging at 15,000 rpm for 3 minutes. Checking that a small white pellet was visible, the isopropanol was then gently poured off into a waste beaker before 200µl of 70% ethanol was added. The tube was inverted a few times to wash the pellet, before centrifuging again at 15,000 rpm for 3 minutes. The ethanol was carefully removed with a pipette due to the possibility of the pellet being loose. The DNA was re-hydrated by adding 25µl of double-distilled H_2O before incubating at 65ºC for one hour.

2.8.2 Polymerase chain reaction

A Master Mix was prepared in a 1.5ml Eppendorf tube comprised of the following components (volumes correspond to 1 DNA sample): 2µl 10x Dream Taq Green Buffer (Thermo scientific, USA), 0.4µl dNTP (Roche, Switzerland) 1µl Primer 1, DARC

KO 1 (sequence 5' – GCT AGA TGC CCT GAC TGT CC – 3', Integrated DNA Technologies, UK), 1µl primer 2, DARCKO 2 (sequence 5' – CCA GTA GCC CAG GTT GCA TA – 3', Integrated DNA technologies, UK), 1µl Primer 3, DARC NEO (sequence 5' – TAT GGC GCG CCA TCG ATC TC – 3', Integrated DNA technologies, UK), 0.1µl DreamTaq DNA Polymerase (Thermo scientific), 13.5µl double-distilled H_2O and 1µl sample DNA. The tube was inverted to mix and span down for 1 second using a mini desktop centrifuge. 19µl of Master Mix and 1µl of DNA sample was pipetted into a 0.2ml PCR tube (Starlab, USA), which was repeated for the number of samples. 1µl of a DNA sample taken from a heterozygous DARC knockout mouse ($DARC^{+/-}$) was used as a positive control, as well as a 1µl DNA sample taken from a wild-type mouse. 1µl double-distilled H_2O was used as a negative control to test for contamination. The PCR lids were placed over tubes, before inverting to mix and spinning down for 1 second. The tubes were placed into the PCR machine (Bio Rad T100 Thermal Cycler) and the PCR program ran as follows: step 1 initial denaturation - 94ºC for 2 minutes, step 2 denaturation - 94ºC for 30 seconds, step 3 annealing - 60ºC for 30 seconds, step 4 extension - 72ºC for 1 minute (steps 2-4 were repeated 32 times), step 5 final extension - 72ºC for 3 minutes and step 6, infinity hold at 10ºC.

2.8.3 Gel electrophoresis

The agarose gel was made by adding 3g of agarose (Melford, UK) to 150ml of 1x TAE buffer (10mM Tris PH 7.4, 1mM EDTA) in a conical flask and swirled to mix. The solution was heated in a microwave for 2-3 minutes, stirring regularly, until the solution was clear. The solution was left to cool for 5 minutes before adding 10µl of SYBR safe DNA gel stain (Invitrogen,) and pouring the gel solution into a gel rack containing a comb divider. The gel was left to set for approximately 30 minutes before the comb divider was carefully removed. The gel and gel rack were transferred to the electrophoresis chamber (Bio Rad Sub-cell GT chamber) and covered with 1x TAE solution. 10µl of 100 base-pair DNA ladder (Invitrogen, Lithuania) was added to the first well for reference, and 10µl of each sample added to the remaining wells. The gel was run at 180V for 40 minutes to allow clear separation of the DNA bands, before the gel was removed from the electrophoresis machine and gel rack, and DNA bands were captured using a Bio Rad Gel Doc EZ imager and visualised using Bio Rad Image Lab software version 5.2.1.

An example of DNA gel staining is presented in figure 2.9. The wild-type DARC allele was detected by amplification of a 400 base pair PCR product, using DARCKO1 (primer 1) and DARCKO2 (primer 2) primers that flank the DARC coding exon. Primer 1 is the sequence that is deleted in the correctly target allele, and primer 2 is complementary to the sequences in exon 2. Primer DARC NEO (primer 3) is complementary to a sequence withing the inserted *neo* gene. DARCKO 2 and DARC NEO were used to amplify a 300 base pair fragment from the targeted DARC gene as described in (Dawson *et al.*, 2000). DNA from a DARC$^{-/-}$ individual expresses a 300 bp band whereas the longer DNA strand from a WT animal expresses a 400 bp band. The DARC heterozygous knockout control (DARC$^{+/-}$) expresses two bands, and both the WT and DARC$^{-/-}$ weight.

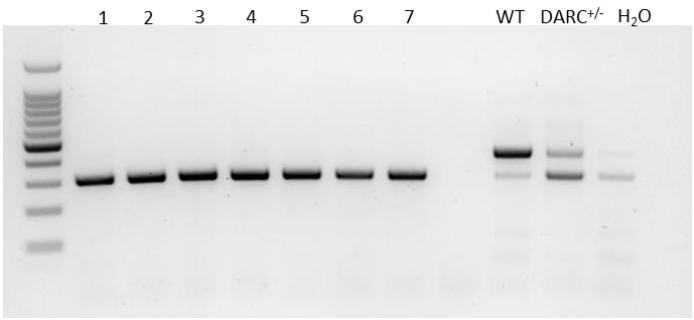


Figure 2.9 Example of DARC$^{-/-}$ genotyping PCR. Lanes 1-7 represent DNA samples taken from weaned DARC$^{-/-}$ pups, expressing a single band at 300 base pairs (bp). The wild-type (WT) control represents DNA from a C57Bl/6J male mouse, expressing a single band at 400bp. The DARC$^{+/-}$ control expresses a band at 400bp and 300bp.

2.9 Immunofluorescence Immunohistochemistry

2.9.1 Slice preparation for immunohistochemistry

Following the completion of electrophysiology measurements, 450µM brain slices were immediately removed from the recording chamber and were stored into a fixative solution of 4% paraformaldehyde (PFA) in Phosphate Buffered Saline (PBS). Slices from WT mice and DARC$^{-/-}$ mice were saved from both juvenile and adult cohorts. In addition, slices that were left to circulate in ACSF and slices incubated with CCL2 were stored from both genotypes and age cohorts.

Slices were removed from PFA and cryoprotected with sucrose solutions. This involved a 4 hour incubation in 10% sucrose in PBS, followed by overnight incubation in 30% sucrose in PBS. Slices were then placed in plastic moulds before being immersed in optimum cutting temperature compound (OCT) (VWR International Ltd., Leicestershire, UK), being careful to remove any bubbles. The moulds and contents were then snap frozen in iso-pentane (2-Methylbutane) (Honeywell, Germany) that has been pre-chilled on dry ice for 20 minutes. The box was submerged for approximately 1 minute, or until the OCT had formed a white solid before being transferred and stored at -70ºC.

Slices were removed from storage and re-sectioned using a Cryostat (Leica CM 1950) at -20ºC. The slices were left to equilibrate to temperature for 20 minutes before re-sectioning to 30µM slices and stored in phosphate buffer with 0.05% Azide.

2.9.2 Immunohistochemistry

All immunohistochemistry (IHC) stages were conducted on free-floating brain sections at room temperature using a 12-well plate netwell system. 3-4 tissue slices were added to each well.

Firstly, optimisation of the anit-Iba1 primary antibody, rabbit (Wako) was conducted at concentrations 1:500, 1:1000 and 1:2000 – a concentration of 1:1000 was found to produce optimal staining.

Tissue sections were washed 3 times in PBS for 5 minutes before permeabilising the membrane by soaking in PBS + TX for 10 minutes. This was followed by a blocking stage to prevent antibody unspecific binding and reducing background staining, by incubating sections in 3% normal goat serum (NGS) in PBS + 0.2% Triton-X (PBS-TX) for 60

minutes. Sections were then incubated with the primary antibody cocktail of anti-Iba1 (rabbit 1:1000, Cat# 019-19741, Wako), anti-NeuN, (guineapig, 1:1000, Cat# ABN90, Sigma) in a solution PBS-TX and 1% NGS overnight at 4ºC. The primary antibody solution was removed, and slices were washed 3 times in PBS for 5 minutes. Slices were next incubated with a secondary antibody cocktail of goat anti-rabbit Alexa Fluor 488 (1:1000 concentration, Cat# A11008, Invitrogen) and goat anti-guineapig Alexa Fluor 546 (1:1000 concentration, Cat# A11074, Life Technologies) in a solution of 1% NGS in PBS at room temperature, in the dark, for 2 hours. After incubation the slices were washed twice in PBS for 5 minutes, before being washed in Hoechst nuclear stain in PBS (1:1000, 10mg/ml stock, Molecular Probes) for 5 minutes. Slices were washed twice more in PBS for 5 minutes, before a final wash in PB to remove any remaining salts.

Slices were mounted onto glass microscope slides (StarFrost, Germany) and covered with borosilicate glass coverslips (VWR, Leicestershire, UK) using Fluoromount aqueous mounting medium (Sigma Aldrich, UK). The slides were kept in the dark and left to dry for 1 hour before storing at 4ºC.

Iba1, NeuN and nuclear stain was z-stack imaged using a Zeiss Confocal LSM 880 at a x20 and x63 oil objective. Z stacks were comprised of 35-45 slices at a x63 objective, and of 10-20 slices at a x20 objective. The average thickness of each hippocampal slice was 9.3-9.8 µm, and images were captured at a resolution of 1024 x 1024 pixels using a pinhole size of 1 AU (Airy Unit).

2.9.3 Microglia analysis

2.9.3.1 Sholl analysis

To analyse microglia branching and morphology, Sholl analysis was conducted on z-stack maximum intensity projection (MIP) images captured by a confocal microscope at a x63 objective. MIP images are an overlay of all z-stack frames and show microglia branching in 3 planes. Sholl analysis could not be directly conducted on MIP images due to the resolution quality, therefore microglia branches were traced using the Simple Neurite Tracer plugin (version 4.0.3) in the ImageJ software (National Institute of Health, USA), as represent in figure 2.10 C. Sholl analysis was then performed on traced images using the Sholl analysis plugin, setting 2µM radius step size and polynomial best fit

parameters (Fig 2.10 D). Maximum branch length, cell branching density known as a ramification index, and the number of branching intersections were measured.

2.9.3.2 Microglia cell count

Microglia proliferation was investigated by counting microglia cell number from MIP images captured using a confocal microscope at a x20 objective. This was conducted by hand, using a multi-point counting tool in the ImageJ software to ensure that cells were not counted more than once. Microglia number was counted from the hilus and cortical subregions on the dentate gyrus.

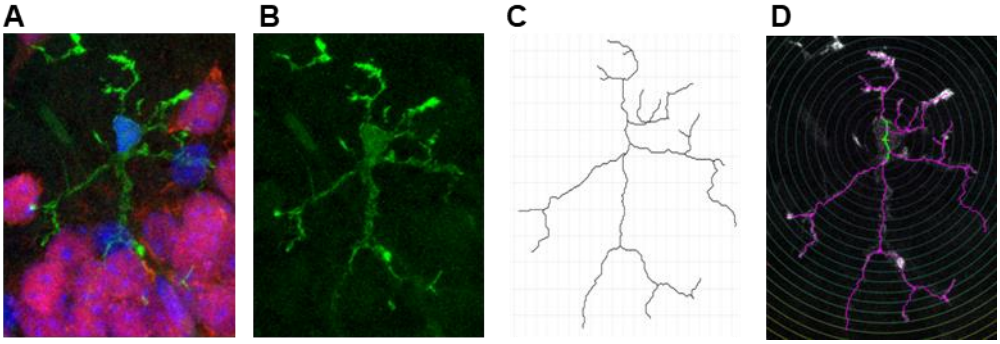


Figure 2.10 Pipeline of neurite tracing and sholl analysis of an Iba1 stained hippocampal microglia cell. (**A**) Cropped maximum intensity projective image of Iba1, Neu-N and Hoechst-nuclear stained microglia cell captured at a x63 objective. (**B**) Channels were split so only alexaflour-488 Iba1 staining was visible. (**C**) Neurite tracing was performed of the Iba1 stained microglia cell. (**D**) Sholl analysis was performed of the traced microglia, with analysis radii set to 2µM.

2.9.4 RNA sequence analysis

To investigate DARC expression localisation in brain cells, an open source database called the Tabula Muris database was utilised to examine single-cell RNA sequencing data. The Tabula Muris database is a compendium of single cells transcriptomic data from mice, comprising of more than 100,000 cells from 20 organs and tissues. Single-cell RNA sequency was performed using two methods: FACS-based cells captured in plates, and microfluidic droplet-based capture (Schaum *et al.*, 2018). The database was screened for DARC localisation and expression on myeloid and non-myeloid cells from the cortex, hippocampus, cerebellum and striatum of 10-15 week old mice, and data was presented as gene counts log-normalised for each cell using the natural logarithm of 1+ counts per million. This database displays data outcomes graphically, using violin plots to highlight data distribution. Raw data of expression levels and statistical detailing is not provided, therefore it is not possible to perform statistical analysis on expression levels.

2.9.5 Statistical analysis

Statistical analysis throughout this thesis was performed using GraphPad Prism 9 (San Diego, California, USA). For electrophysiological experiments, an N number represents recordings from a single brain slice from one animal.

2.9.5.1 Two Way ANOVA

Stimulus response recordings were analysed using a Two Way ANOVA and the Sidak multiple comparisons test. A Two Way ANOVA was selected as the analysis required examining the effect of two factors (age or genotypes and stimulation input) against a dependent variable, fEPSP slope. The Sidak correction was selected for this data type as it has the highest power and acts to control family-wise error rate, which is the probability of making one or more false discoveries when performing multiple hypothesis tests. A Sidak test is also required when one of the variables has a selected set mean, such as the stimulus input, which is a pre-set value. A Two Way ANOVA with Sidak multiple comparisons was also used to statistically analyse the intracellular recording voltage-current curves, as the current input was also a pre-set value.

For experiments investigating the effects of chemokines (CCL2 and CCL5) and chemokine receptor antagonists (RS504393 and SB328437) on perforant pathway activity, a Two Way ANOVA and Tukey multiple comparisons test was used. This post-hoc test was selected as pairwise comparisons between group means wanted to be investigated. Microglia cell count analysis and mouse behavioural analysis (open field maze and y-maze experiments) was also performed using this statistical test.

2.9.5.2 Mann Whitney test

When comparing the differences between two independent data groups, a Mann-Whitney test was utilised as this statistical test is non-parametric, and does not assume normal distribution between data groups.

Chapter 3: Age-dependent changes in perforant pathway activity

3.1 Introduction

It is well documented that hippocampal physiology and function changes with normal aging. A decline in hippocampal-dependent episodic memory, spatial learning and working memory has been observed in aged individuals in behavioural experiments using rodent (Gage *et al.*, 1984; Shen *et al.*, 1997), primate (Rapp, Kansky and Roberts, 1997) and human models (Newman and Kaszniak, 2000; Moffat, Zonderman and Resnick, 2001). In addition, changes in hippocampal excitability have been observed, with synaptic transmission and expression of synaptic proteins reduced in aged individuals, contributing to age-related cognitive defects (Wang *et al.*, 2007; Gureviciene, Gurevicius and Tanila, 2009). Aging can be defined as the progressive deterioration of physiological function with increasing age (Galloway, 1993). However, most studies examining the effects of aging focus only on the comparison between young subjects with the very old. For example, rodent studies often use animals that are at least 18 months of age, and human studies often use people over the age of 60. Studies have shown, however, that aging may be progressive. For example, hippocampal-dependent spatial memory and object recognition is lower in middle-aged adults compared to young adults (Williams *et al.*, 2019; Stark, Yassa and Stark, 2010). Moreover, a decline in memory function in middle age has been associated with elevated risk of Alzheimer's disease onset (Ritchie *et al.*, 2018). Despite these findings, few studies have examined physiological changes during early aging. In this chapter I investigated whether the hippocampus exhibits physiological changes along perforant pathway in early aging. I hypothesised that small changes in hippocampal excitability would be detected as changes in hippocampal excitability has been previously observed in middle-aged mice.

3.2 Results

3.2.1 Age-dependent changes in perforant pathway activity

Several studies examine the effects of late aging on perforant pathway physiology, with loss of dentate gyrus synapses reported in rodent late aging (Geinisman *et al.*, 1992). Neurophysiology studies show that entorhinal cortex to dentate gyrus long-term potentiation is substantially reduced in old rodents, and stimulation of perforant pathway afferents results in greater dentate gyrus quantal size in aged rats (Foster *et al.*, 1991; Barnes, Rao and Houston, 2000). These studies examine the effects of late aging on perforant pathway physiology, comparing rodents between 3-12 months of age and 24-

35 months of age, but little is known about how hippocampal function changes during early aging. I therefore examined how early aging affects the excitability of the perforant pathway *in vitro*. Hippocampal slices were prepared from juvenile (9-15 weeks old) and adult (25-35 weeks old) wild-type mice. Once slices had acclimatised to the *in vitro* conditions, the perforant pathway was activated by electrically stimulating the entorhinal cortex layer II from 5V to 100V and subsequently generated field EPSPs (fEPSPs) were recorded from the dentate gyrus. The rising slope of fEPSPs were measured and plotted against entorhinal cortex stimulation input. The supra-pyramidal and infra-pyramidal dentate gyrus subregions have previously been described to contain different circuit functions (Scharfman *et al.*, 2002); I therefore measured fEPSPs separately from both subregions.

fEPSP slope measured from the supra-pyramidal dentate gyrus were significantly greater in adult mice compared to juvenile mice when the entorhinal cortex was stimulated at 70V-100V. (70V: juvenile 0.563 ± 0.099V vs adult 2.160 ± 0.683V, P=0.036. 80V: juvenile 0.640 ± 0.098V vs adult 2.404 ± 0.755V, P=0.013. 90V: juvenile 0.729 ± 0.093V vs adult 2.788 ± 0.751V, P=0.0019. 100V: juvenile 0.742 ± 0.093V vs adult 2.822 ± 0.777V, P=0.0017, N=6 mice. Fig 3.1). fEPSPs measured from the infra-pyramidal dentate gyrus were also significantly larger in adult mice compared to juvenile mice when the entorhinal cortex was stimulated at 90V-100V (90V: juvenile 0.576 ± 0.099V vs adult 1.343 ± 0.294, P=0.0065. 100V: juvenile 0.592 ± 0.110V vs adult 1.466 ± 0.339V, P=0.0011, N=6 mice**.** Fig 3.1), however to a much lesser extent than observed in the supra-pyramidal dentate gyrus (Fig 3.1 C). These data indicate that dentate gyrus excitability increases when aging from juvenile to adulthood, with the supra-pyramidal subregion showing more pronounced changes than the infra-pyramidal subregion.

The perforant pathway projects sensory inputs from the entorhinal cortex to the dentate gyrus and then via the mossy fibres to the CA3 (Steward, 1976). As dentate gyrus excitability was affected in early aging, I next examined whether neuronal excitability in the CA3 was also affected. The experimental setup was carried out as described above, however fEPSPs were measured from the CA3 subregion. There was no significant difference in CA3 fEPSPs observed between juvenile and adult mice (Fig 3.2). Hence, age-dependent hippocampal excitability is regionally specific to the dentate gyrus and is not persistent along the perforant pathway.

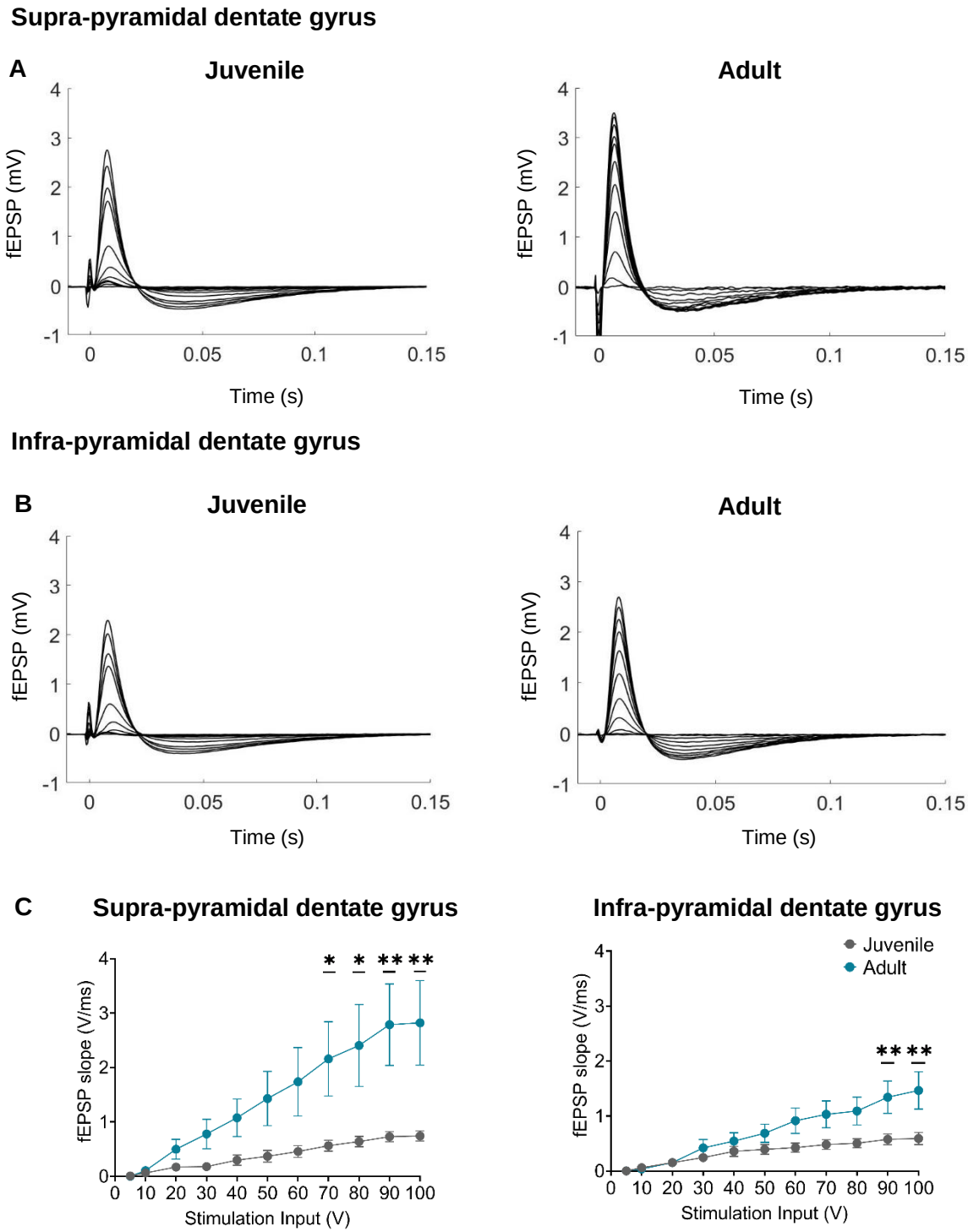


Figure 3.1 Increased excitability of the dentate gyrus in adult mice compared to juvenile mice varies across dentate gyrus subregions. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex. **(A–B)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal (A) or infra-pyramidal blade (B) of the dentate gyrus in juvenile or adult mice. The entorhinal cortex was stimulated at time 0. fEPSPs represent the average response of 10 electrical stimulations for each intensity (5V–100V). **(C)** Stimulus response curves showing the slope of fEPSPs as a function of stimulation intensity in juvenile or adult mice. Bars represent mean ± SEM. * P ≤ 0.05, ** P ≤ 0.01, two-way ANOVA with Sidak multiple comparison tests. N=6 mice for each study group (N number represents 1 hippocampal slice recording per animal).

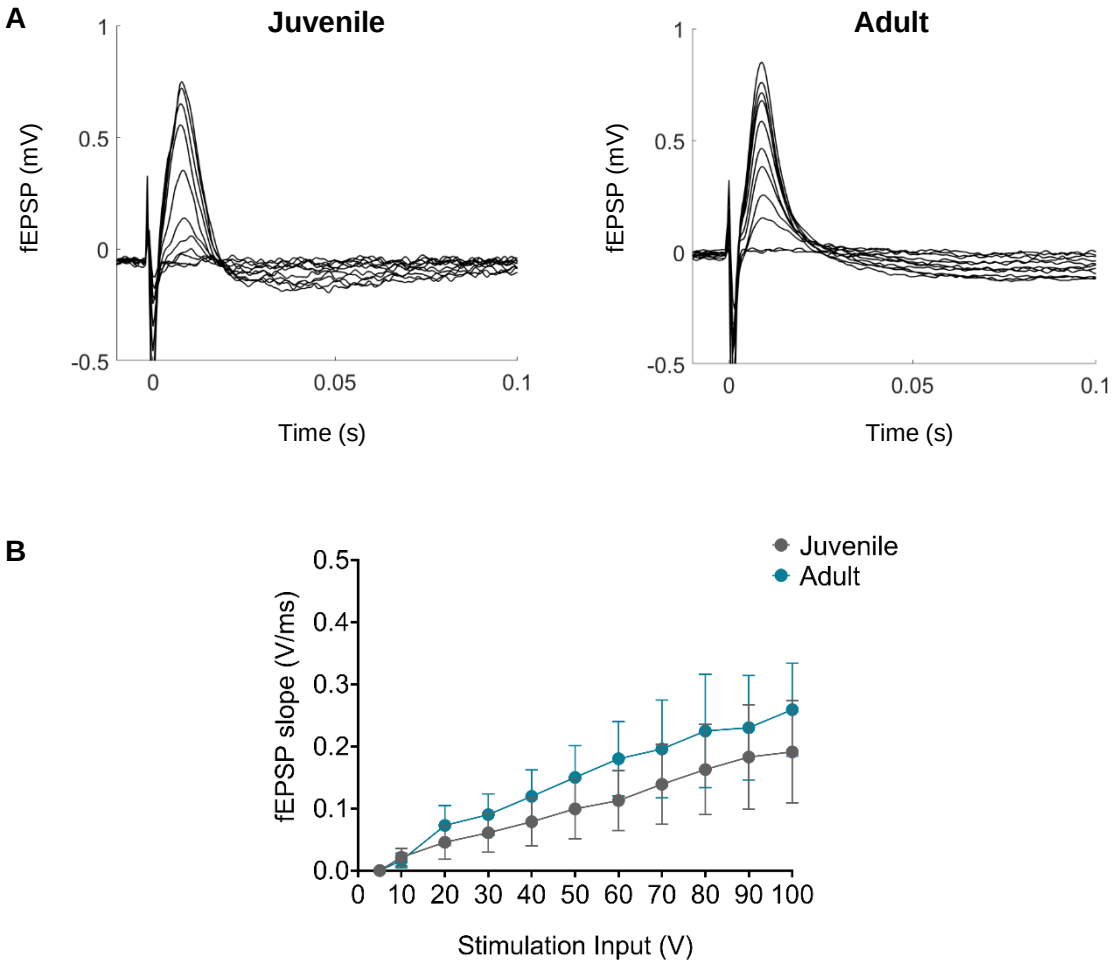


Figure 3.2 Aging has no effect on CA3 excitability. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex. **(A)** Representative field EPSPs (fEPSPs) recorded from the CA3 in juvenile or adult mice. The entorhinal cortex was stimulated at time 0. fEPSPs represent the average response of 10 electrical stimulations (1 Hz) for each intensity (5V–100V). **(B)** Stimulus response curve showing the slope of fEPSPs as a function of stimulation intensity in juvenile or adult mice. Bars represent mean ± SEM. Two-way ANOVA with Sidak multiple comparison tests. N=6 and N=7 mice for juvenile and adult groups respectively (N number represents 1 hippocampal slice recording per animal).

3.2.2 Intracellular recordings of dentate gyrus granule cells

This far I have recorded changes in field EPSPs, however in order to understand the mechanisms underlying these changes I next examined if the age-dependent extracellular changes in dentate gyrus excitability seen in 3.2.1 were due to changes in dentate gyrus granule cellular properties. Hippocampal slices were prepared from juvenile (9-15 weeks old) and adult (25-35 weeks old) wild-type mice and intracellular properties were measured *in vitro* from supra-pyramidal and infra-pyramidal dentate gyrus granule cells.

I first examined whether early aging affected granule neurone EPSP strength upon perforant pathway stimulation. The perforant pathway was activated by electrical stimulation of the entorhinal cortex layer II at 50% maximum and 100% maximum stimulation, and subsequent EPSPs were measured from individual supra-pyramidal and infra-pyramidal dentate gyrus granule neurons in juvenile and adult mice.

There were no age-related differences in granule neurone EPSP amplitude measured from the supra-pyramidal subregion (50% maximum stimulation: juvenile 13.04 ± 2.373µV, n=7 cells vs adult 20.43 ± 2.317µV, n=13 cells, ns. 100% maximum stimulation: juvenile 13.011 ± 2.964µV, n=7 cells vs adult 18.50 ± 2.345µV, n=13 cells, ns. Fig 3.3) or infra-pyramidal subregion (50% maximum stimulation: juvenile 11.98 ± 2.611µV, n=5 cells vs adult 11.17 ± 1.594µV, n=9 cells, ns. 100% maximum stimulation: juvenile 11.06 ± 1.951µV, n=5 cells vs adult 11.16 ± 2.087µV, n=10 cells, ns. Fig 3.4).

However, when directly comparing subregional differences in EPSP amplitudes, supra-pyramidal granule neurons in adult mice, exhibit larger EPSP amplitudes upon perforant pathway stimulation compared to infra-pyramidal granule neurons. (50% maximum stimulation: supra-pyramidal 20.43 ± 2.317µV, n=13, vs infra-pyramidal 11.17 ± 1.594µV, n=9, P=0.0129. 100% maximum stimulation: supra-pyramidal 18.50 ± 2.345µV, n=13, vs infra-pyramidal 11.16 ± 2.087, n=10, P=0.0475, Fig 3.5 B). There are no significant differences observed between the two subregions in juvenile mice (Fig 3.5 A). These data suggest that postsynaptic responses do not underlie the age-dependent changes in hippocampal excitability observed in Fig 3.1. A caveat of this experiment is that EPSP amplitude is dependent on the resting membrane potential. Any significant changes in resting membrane potential, will affect EPSP closeness to the reversal potential.

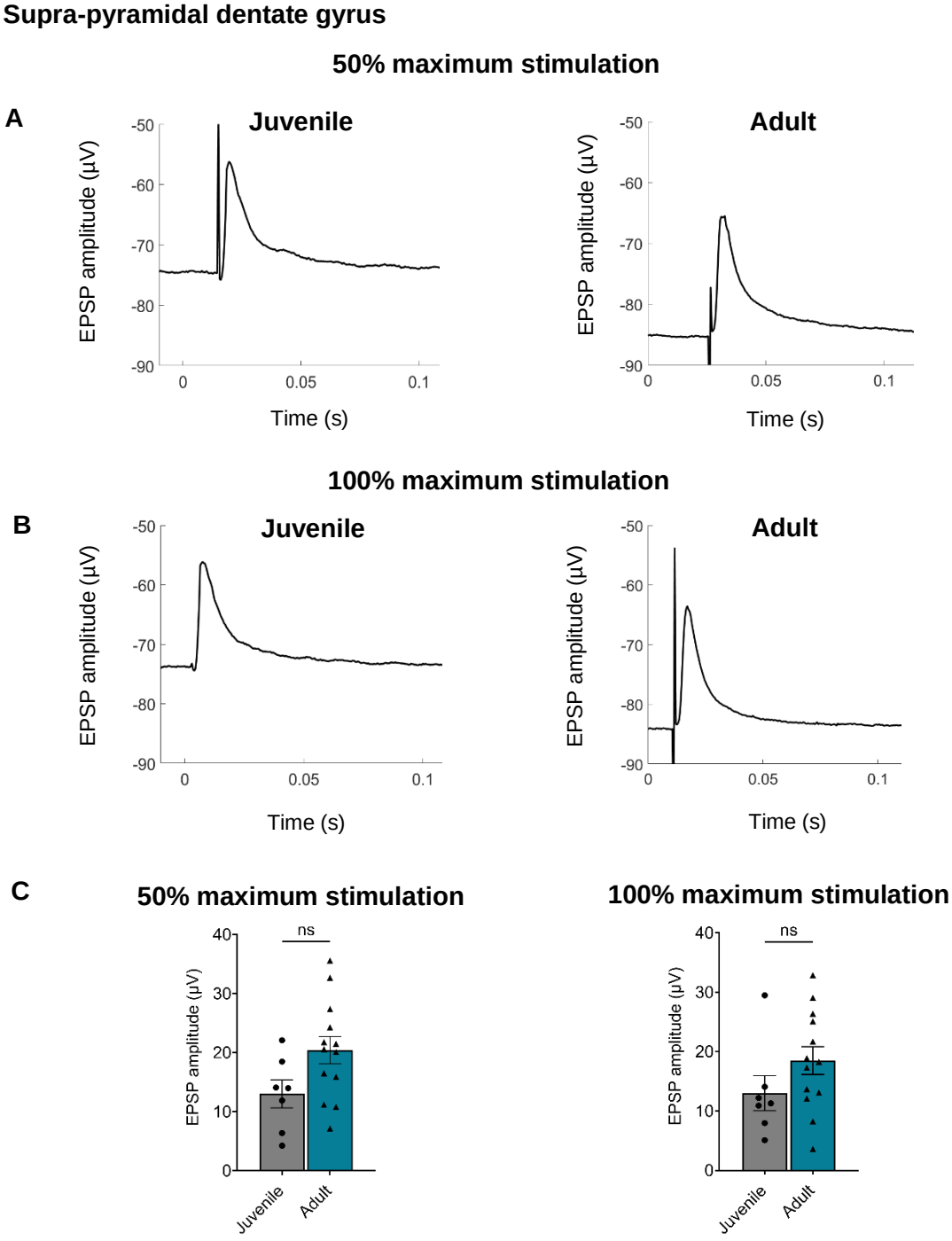


Figure 3.3 Aging has no effect on EPSP amplitude in supra-pyramidal dentate granule neurons. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex at 50% or 100% maximum stimulation. (**A–B)** Representative EPSP traces from the supra-pyramidal blade of the dentate gyrus in juvenile or adult mice stimulated at 50% maximum voltage (A) and 100% maximum voltage (B). **(C)** Grouped data of the mean average EPSP amplitude stimulated at 50% maximum stimulation or 100% maximum stimulation recorded from supra-pyramidal granule cells in juvenile and adult wild-type mice. Bars represent mean ± SEM (Mann-Whitney test). N=7 and N=13 cells for juvenile and adult mice respectively (N number represents 1 hippocampal slice recording per animal).

Infra-pyramidal dentate gyrus

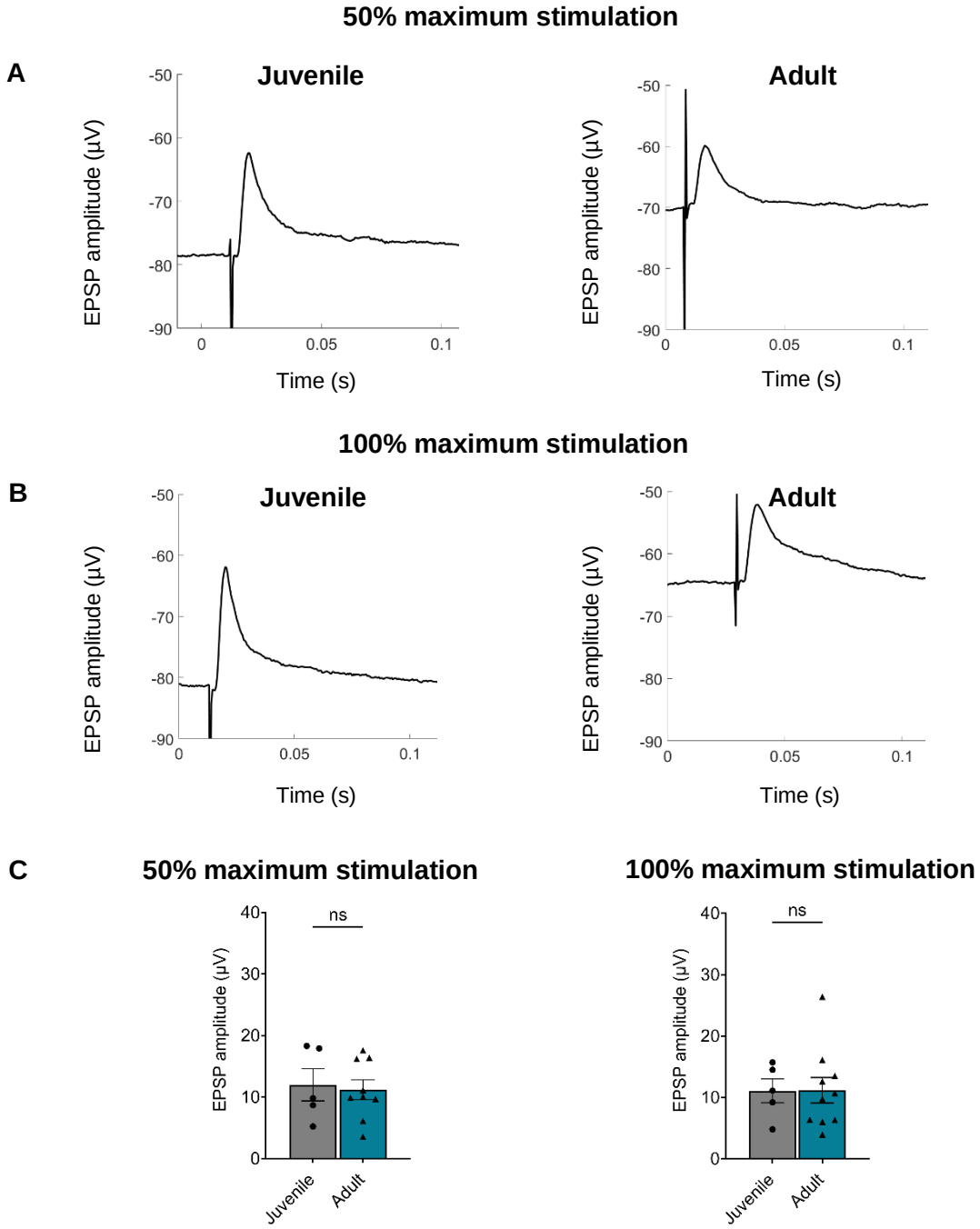


Figure 3.4 Aging has no effect on EPSPs amplitude measured from infra-pyramidal dentate granule neurons. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex at 50% or 100% maximum stimulation. (**A–B)** Representative EPSPs recorded from the infra-pyramidal blade of the dentate gyrus in juvenile and adult mice stimulated at 50% maximum voltage (A) and 100% maximum voltage (B). **(C)** Grouped data showing the mean average EPSP amplitude stimulated at 50% maximum stimulation and 100% maximum stimulation recorded from infra-pyramidal granule cells in juvenile and adult wild-type mice. Bars represent mean ± SEM (Mann-Whitney test). N=5 and N=10 cells for juvenile and adult mice respectively (N number represents 1 hippocampal slice recording per animal).

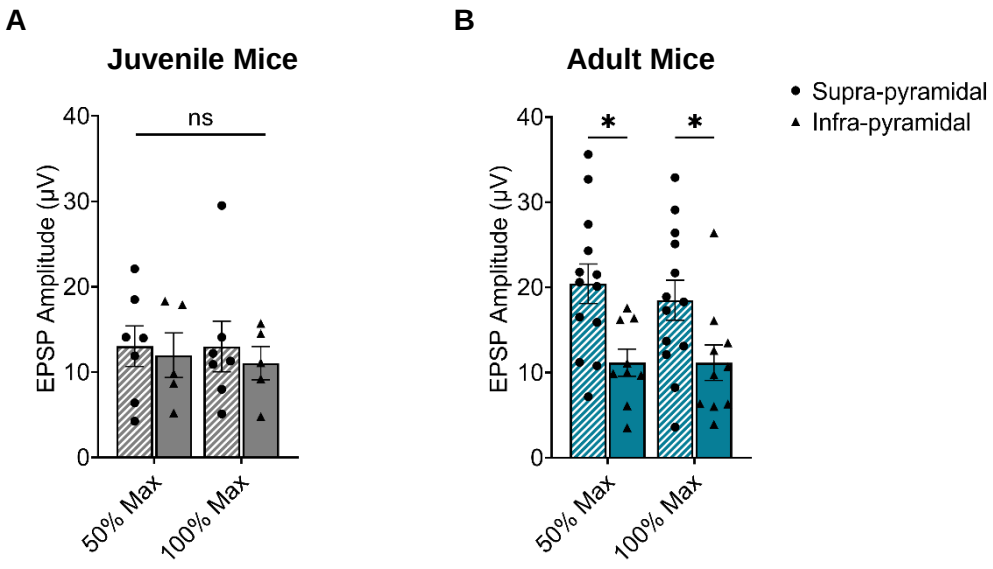


Figure 3.5 EPSPs are larger in supra-pyramidal dentate granule neurons than infra-pyramidal dentate granule neurones in adult mice. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex at 50% or 100% maximum stimulation. Grouped data showing the mean average EPSP amplitude stimulated at 50% maximum stimulation and 100% maximum stimulation recorded from supra-pyramidal and infra-pyramidal granule cells in juvenile (**A**) and adult (**B**) wild-type mice. Bars represent mean ± SEM. * P ≤ 0.05, two-way ANOVA with Sidak multiple comparison test.

As the postsynaptic response of granule neurons was not responsible for the age-dependent increase in dentate gyrus excitability, I next examined whether resting state properties of supra-pyramidal and infra-pyramidal granule cells could be underlying the extracellular differences observed.

I first studied the membrane resistance of supra-pyramidal and infra-pyramidal granule neurons from juvenile and adult mice. This was achieved by piercing a granule neuron and injecting input current step-wise from -0.5 nA to +0.5 nA (Fig 3.6 A and B) and measuring the subsequent voltage change in membrane potential. Current – voltage curves were plotted (Fig 3.6 C) and Ohm's law was used to calculate membrane resistance.

Supra-pyramidal granule neurons in adult mice had a significantly higher membrane resistance compared to granule neurons in juvenile mice (juvenile mice, 20.04 ± 1.051 MΩ vs adult mice 32.90 ± 1.191 MΩ, P<0.0001), however no significant difference was

observed in infra-pyramidal granule neurons (juvenile mice 24.20 ± 1.579 MΩ vs adult mice 27.44 ± 1.685 MΩ, ns Fig 3.6 D). These data indicate that early aging affects the membrane resistance of dentate gyrus granule in a subregion specific manner, with only the supra-pyramidal subregion affected.

I next examined whether the resting membrane potential of dentate gyrus granule neurons were affected by early aging. This was conducted by recording the membrane potential from individual granule neurons for 1 minute in the supra-pyramidal dentate gyrus (Fig, 3.7 A) and infra-pyramidal dentate gyrus (Fig 3.7B) in juvenile and adult cohorts. I observed that supra-pyramidal granule neurons had a more negative resting membrane potential in adult mice compared to juvenile mice (juvenile mice -74.11 ± 3.321 mV vs adult mice -85.26 ± 1.325 mV, P=0.0066), however no age-related difference was observed in resting membrane potential in infra-pyramidal granule neurons (juvenile mice -70.75 ± 4.345 mV vs adult mice -68.05 ± 1.598 mV, ns Fig 3.7 D).

These intracellular measurements have highlighted that early aging effects resting properties of granule neurons, with membrane resistance increasing and resting membrane potential being more negative in adult mice compared to juveniles. These changes in fundamental cellular properties could underlie the age-dependent changes in extracellular excitability observed between juvenile and adult mice (Fig 3.1). Furthermore, age-related changes in membrane potential and resistance were exclusive to supra-pyramidal granule neurons, and greater EPSP generation was observed in supra-pyramidal cells compared to infra-pyramidal cells in adult mice upon perforant pathway stimulation (Fig 3.5). These data strongly indicate that granule neurons in the supra-pyramidal dentate gyrus are more effected by aging than those in the infra-pyramidal subregion, which could account for the more prominent changes in hippocampal excitability occurring in the supra-pyramidal region (Fig 3.1).

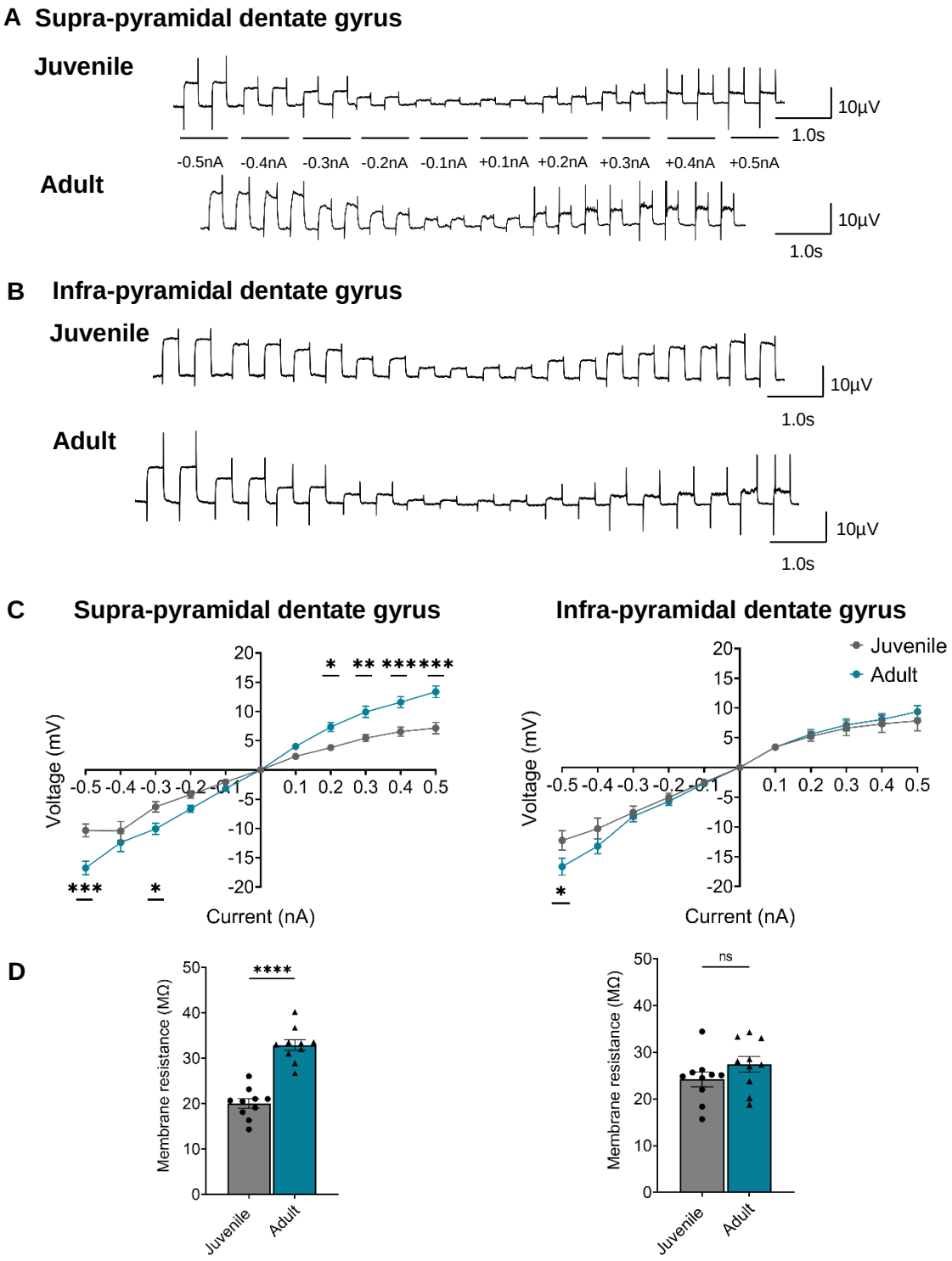


Figure 3.6 Membrane resistance of dentate granule cells increases with aging in a region specific manner. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice and intracellular properties were recorded from supra-pyramidal and infra-pyramidal dentate gyrus granule cells. **(A–B)** Representative current-voltage recordings from supra-pyramidal (A) or infra-pyramidal (B) granule cells from juvenile or adult mice. An input current was injected into cells step wise from -0.5 - +0.5 nA. **(C)** Current-voltage curves showing the voltage output (mV) in response to stepwise input currents (nA). (**D**) Grouped data showing membrane resistance of supra-pyramidal (left image) or infra-pyramidal (right image) granule neurones in juvenile and adult mice. Bars represent mean ± SEM * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P < 0.0001, (C) two-way ANOVA with Sidak multiple comparison tests, (D) Mann-Whitney test. N=10 cells for each group recorded from 5 juvenile mice, and 5 adult mice.

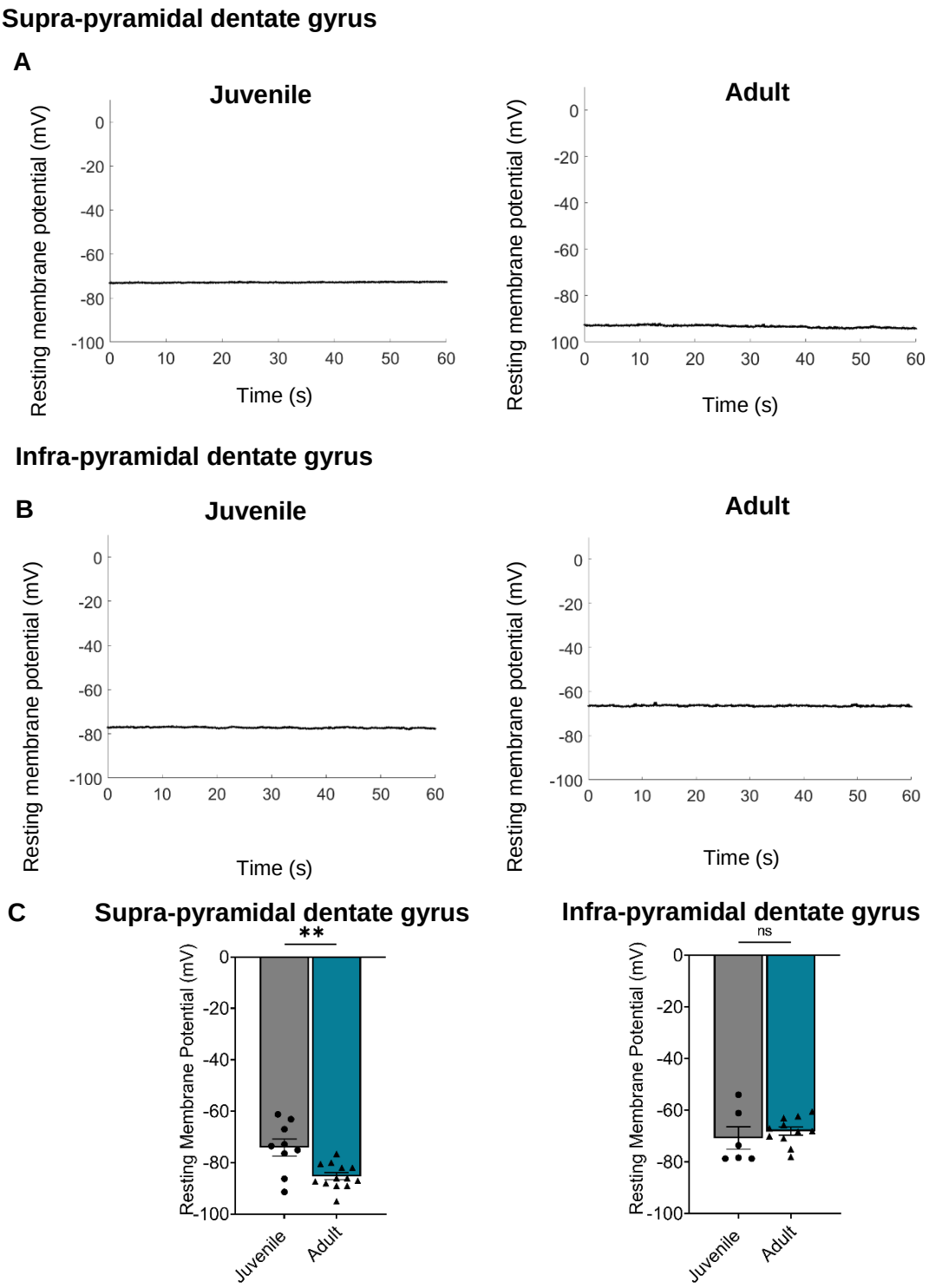


Figure 3.7 Resting membrane potential of supra-pyramidal dentate gyrus granule neurons is hyperpolarised in adult mice compared to juveniles. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice and resting membrane potential was recorded from supra-pyramidal and infra-pyramidal granule cells of the dentate gyrus. **(A–B)** Representative resting membrane potential traces from supra-pyramidal (A) or infra-pyramidal dentate gyrus granule cells (B) from juvenile or adult mice. (**C**) Grouped data showing resting membrane potential of supra-pyramidal or infra-pyramidal granule neurones in juvenile and adult mice. Bars represent mean ± SEM ** P ≤ 0.01, Mann-Whitney test. N=9 and N=13 supra-pyramidal cells for juvenile and adult mice respectively, and N=6 and N=11 infra-pyramidal cells for juvenile and adult mice respectively (recorded from 5 juvenile mice, and 5 adult mice)

3.2.3 Early aging does not affect the cortical-hippocampal gamma network

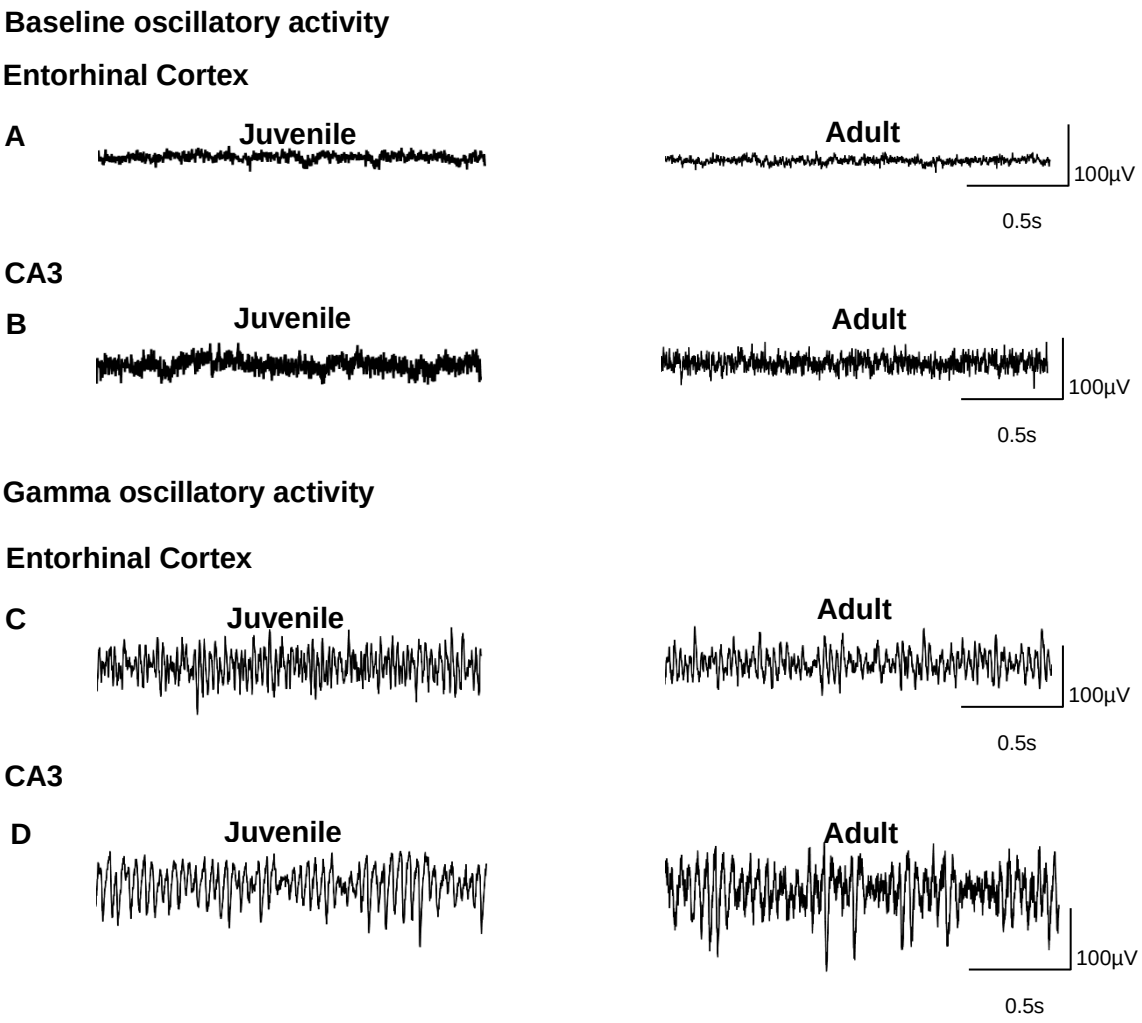
My data thus far indicates that early aging increases dentate gyrus excitability, and this likely occur through changes in the intrinsic membrane properties of granule cell neurons (Fig 3.1 – 3.7). The dentate gyrus is part of a complex cortical-hippocampal network that is required for information processing through temporal co-ordination of the activity of several neurons in the circuit, which manifest as oscillations local field potentials. Therefore, I next examined whether early aging affected hippocampal networks in the cortical-hippocampal environment by measuring gamma oscillations *in vitro*.

Gamma oscillations are abundant in most behavioural states but are particularly prevalent during primary sensory processing during wakefulness (Gray and Singer, 1989). Hippocampal gamma oscillations are important in memory encoding, working memory and spatial navigation (Newman *et al.*, 2013; Yamamoto *et al.*, 2014; Bieri, Bobbitt and Colgin, 2014), and power and frequency of the gamma rhythm has been shown to decrease in aged human subjects (Murty *et al.*, 2020). I therefore felt it was important to investigate if hippocampal gamma was affected in early aging. The entorhinal cortex supplies the major neocortical input from a number of sensory regions to the hippocampus via the perforant pathway (Witter *et al.*, 1989) and has a pivotal role in gating information flow from the neocortex to the hippocampus (Fernandez *et al.*, 1999; Fell *et al.*, 2001). It has also been shown to undergo pathologic changes that occur in memory function disorders such as Alzheimer's Disease (Hyman *et al.*, 1984; Braak and Braak, 1991), highlighting the region's importance in memory-related pathophysiology.

I have shown that dentate gyrus granule cell properties are affected in early aging. This region was investigated as the dentate gyrus plays a pivotal role in routing sensory information and reducing interference along the perforant path between the entorhinal cortex and CA3 and undergoes significant synaptic loss during late aging (O'Reilly and McClelland, 1994; Geinisman *et al.*, 1992; Steward, 1976). Although oscillatory measurements have been successfully recorded from the dentate gyrus in previous studies (Towers *et al.*, 2002; Pöschel, Draguhn and Heinemann, 2002), in my experimental setup, gamma oscillations measured from the dentate gyrus were coupled with spiking activity, and gamma oscillations did not stabilise within a 6 hour period

(stable gamma was achieved when the peak power of the oscillation did not change by more than 10% in a 30-minute window). Therefore, I instead examined whether early aging was affecting the synchrony of oscillations independently generated in the entorhinal cortex and CA3. As I have previously shown that excitability in the dentate gyrus increases in early aging, likely to occur through changes in intrinsic membrane properties, I predicted that these changes could potentially affect hippocampal network oscillations and result in a decrease in synchronous oscillatory activity.

Hippocampal slices were prepared from juvenile (9-15 weeks old) and adult (25-35 weeks old) wild-type mice and oscillations were measured simultaneously from the entorhinal cortex and CA3. Control recordings were taken from both subregions after 1 hour of acclimatising to the *in vitro* conditions (Fig 3.8 A and B). 200nM of kainic acid was added to the slices via bath application and induced gamma rhythms were measured from both subregions (Fig 3.8 C and D). Interregional gamma synchrony has been proposed as a mechanism to precisely coordinate upstream and downstream neuronal assemblies (Singer, 1993; Engel, Fries and Singer, 2001; Fries, 2009; Buzsáki and Wang, 2012) and synchronisation of gamma oscillations between the entorhinal cortex and hippocampus, as well as within the hippocampus, has been shown to be associated with successful memory encoding in humans (Fell *et al.*, 2001) and macaques (Jutras, Fries and Buffalo, 2009). Additionally, entorhinal cortex and CA3 regions have increased spike coupling during object learning behaviour (Fernández-Ruiz *et al.*, 2021). I analysed oscillations measuring the phase locking value between the entorhinal cortex and CA3 in juvenile and adult mice and observed that a coupling spike appeared at the gamma frequency (~25 Hz) in both juvenile and adult cohort (Fig 3.8 E), however there was no significant difference in phase locking values between juvenile and adult mice (juvenile mice 0.19 ± 0.038 vs adult mice 0.169 ± 0.029, ns, Fig 3.8 F). These data suggest that phase locking between the entorhinal cortex and CA3 exists at the gamma frequency, however this remains unaffected by early aging.



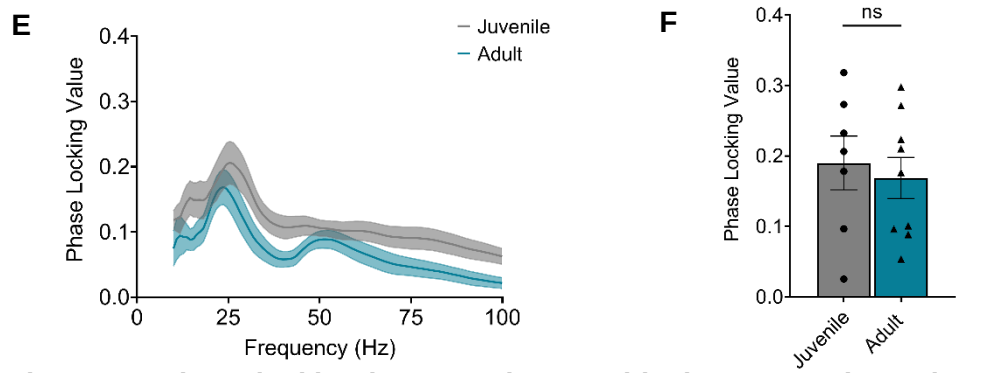


Figure 3.8 Phase locking between the entorhinal cortex and CA3 is not disrupted by aging. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. Gamma oscillations were induced by bathing slices in 200 nM kainic acid and oscillations were recorded from the entorhinal cortex and CA3 in juvenile and adult mice. **(A–B)** Examples of baseline traces recorded from the entorhinal cortex (A) and CA3 (B) in slices bathed in circulating ACSF. (**C-D**) Examples of gamma oscillation traces recorded from the entorhinal cortex (C) and CA3 (D) in juvenile and adult mice. (**E**) Entorhinal cortex and CA3 phase locking values as a function of maximum oscillation frequency in adult and juvenile mice (**F**) Grouped data showing entorhinal cortex and CA3 phase locking values (taken from the peak amplitude of oscillations) in juvenile and adult mice. Bars represent mean ± SEM, Mann-Whitney test. N=7 and N=9 mice per group for juvenile and adult mice respectively (N number represents 1 hippocampal slice recording per animal).

Previous literature has indicated that late aging affects the power and frequency of hippocampal gamma oscillations (Murty *et al.*, 2020); I therefore examined whether the power and frequency of gamma oscillations in the entorhinal cortex and CA3 were affected by early aging. Power spectrums of the recorded oscillations were produced for juvenile and adult mice in the entorhinal cortex (Fig 3.9 A) and CA3 (Fig 3.9B), however no significant differences in gamma oscillation power or frequency were observed between juvenile and adult mice in either subregion (Fig 3.9 C).

These experiments have indicated that early aging affects dentate gyrus excitability upon perforant pathway activation, most likely caused by changes in granule neurone resting cell properties in this subregion. Despite these changes, there was no evident disruption in gamma oscillations between the entorhinal cortex and CA3, suggesting that age-dependent changes in dentate gyrus properties are not affecting the wider neuronal network during early aging.

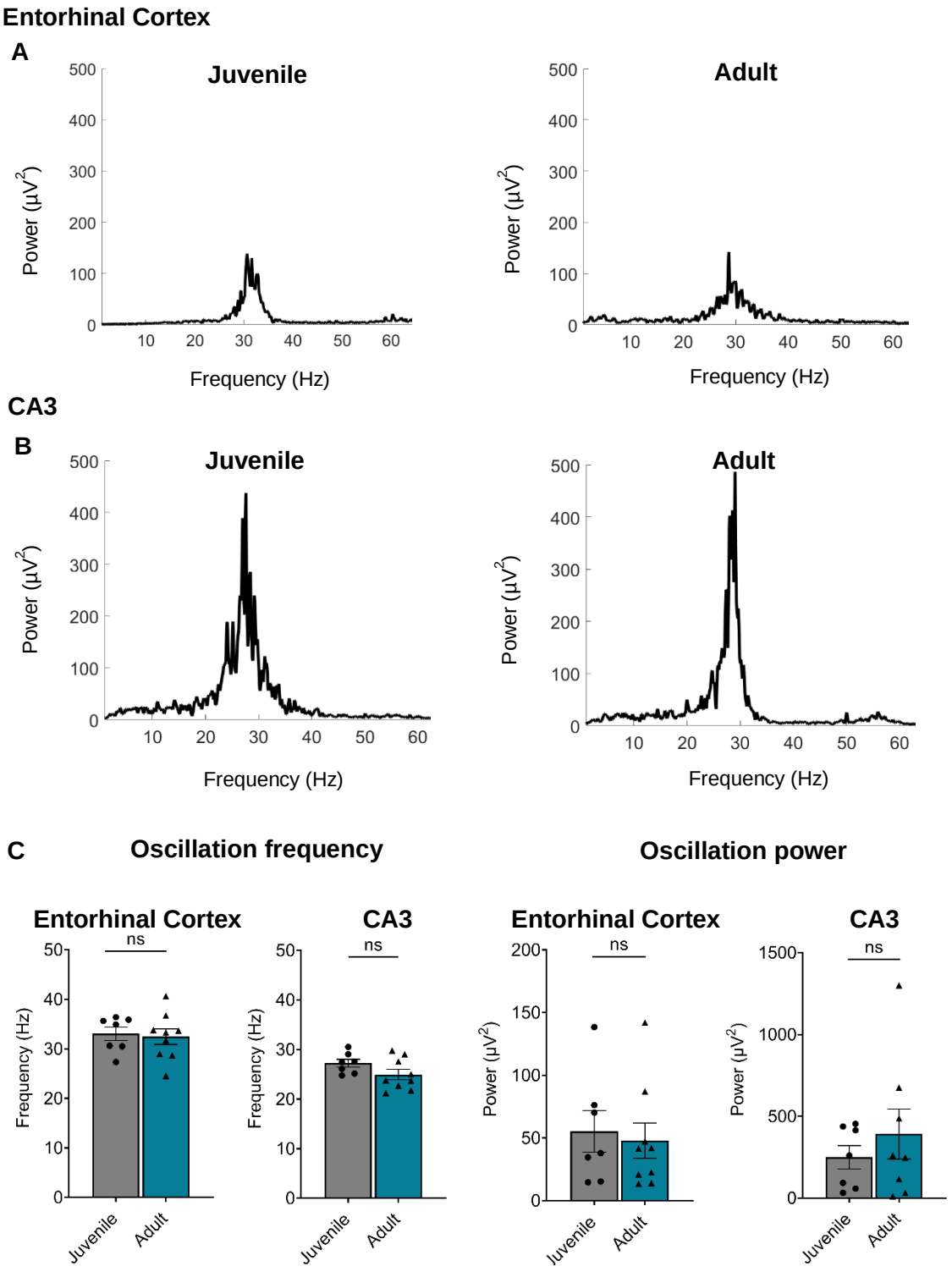


Figure 3.9 Frequency and power of gamma oscillations in the entorhinal cortex and CA3 are not affecting by aging. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. Gamma oscillations were induced by bathing slices in 200 nM kainic acid and oscillations were recorded from the entorhinal cortex and CA3 regions in juvenile and adult mice. **(A–B)** Examples of fast Fourier transforms of gamma oscillations recorded from the entorhinal cortex (A) and CA3 (B) in juvenile and adult mice. (**C**) Grouped data showing oscillation frequency and power recorded from the entorhinal cortex and CA3 in juvenile and adult mice. Bars represent mean ± SEM, Mann-Whitney test. N=7 and N=9 mice per group for juvenile and adult mice respectively (N number represents 1 hippocampal slice recording per animal).

3.3 Discussion

Perforant pathway connectivity and age-dependent changes in hippocampal physiology and function are well characterised. Almost all experimental observations investigate the impact of late aging on hippocampal function, often comparing groups of rodents that are 3-12 months of age versus 24-35 months of age. Aging can be described as the progressive deterioration of physiological function with increasing age (Galloway, 1993), yet there is very little literature examining the effects of early aging and whether physiological aging is also progressive. A spatial memory and object recognition task in humans has highlighted that hippocampal-dependent memory declines between young adults and middle-aged subjects (Williams *et al.*, 2019; Stark, Yassa and Stark, 2010), and a decline in memory function in middle age has been associated with elevated risk of Alzheimer's disease onset (Ritchie *et al.*, 2018). Sparse physiological examinations into early aging and perforant pathway activity have been conducted, despite middle age potentially being a sensitive and critical period for the development of neurodegenerative disorders (Nikodemova *et al.*, 2016).

3.3.1 Age dependent changes in perforant pathway activity

3.3.1.1 Perforant pathway excitability changes in early aging

My results showed that dentate gyrus fEPSPs generated from entorhinal cortex perforant pathway stimulation were larger in adult mice (25-35 weeks old / 6-8months old) compared to juveniles (9-15 weeks old / 2-3 months old), which was significant at higher stimulation intensities, however no significant changes were observed in the CA3. This data suggests that dentate gyrus granule neurons become more excitable to perforant pathway activation with early aging, however this is not translated in CA3 pyramidal neuron excitability.

These results are different to those observed in late aging. Previous work has shown that perforant pathway stimulation leads to decreased EPSP amplitude in granule cells (Barnes, Rao and Houston, 2000a; Froc *et al.*, 2003; Barnes, 1979) and decreased fEPSPs in the dentate gyrus of aged rodents (Barnes and McNaughton, 1980; Froc *et al.*, 2003). These aging-dependent changes in excitability are thought to occur due to synapses, with the dentate gyrus in aged rats reported to receive one-third fewer synaptic contacts from the entorhinal cortex to the dentate gyrus than in young rats (Geinisman *et al.*, 1992; Bondareff and Geinisman, 1976). Moreover, a human study

found that elderly individuals with memory impairments have fewer perforant path synapses (Scheff *et al.*, 2006) caused by reduced axon collaterals from the entorhinal cortex layer II to dentate granule cells (Merrill, Chiba and Tuszynski, 2001).

Together, these data suggest that the excitability of the dentate gyrus may increase as rodents move into adulthood, but then decrease as the rodent transitions to an older age. This is represented schematically in figure 3.10.

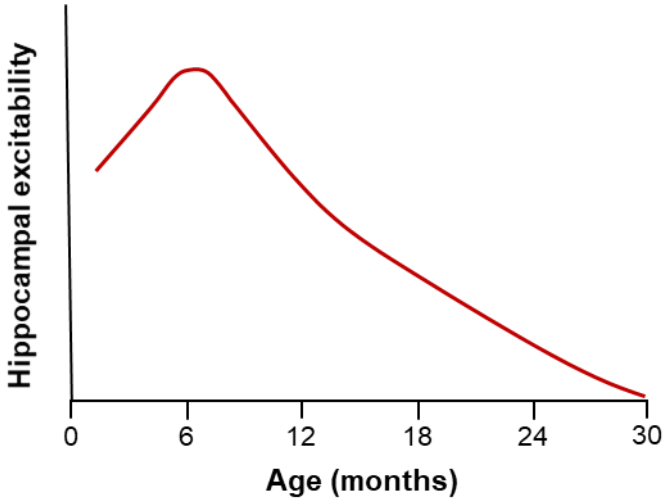


Figure 3.10 Schematic representation of presumed hippocampal excitability changes with aging. I observe that hippocampal excitability increases in early aging (from 2-3 months of age vs 6-8 months of age). Other *in vitro* electrophysiology experiments have indicated that hippocampal excitability is reduced between rodents of 3 months vs 25 months of age, 2-3 months of age vs 10-12 months of age, and 10-12 months of age vs 25-30 months of age (Barnes *et al.,* 1980; Froc et al; 2003 and Schreurs et al., 2017).

Although a bell-shaped curve in hippocampal excitability changes from juvenile to old age has not been previously reported, this phenomenon is not new. For example, motor behavioural experiments have identified a similar pattern of age-related activity, with rats showing a steady increase in motor performance from 4-12 months of age, followed by a faster decline in motor performance from 12 months to 40 months of age (Ernyey *et al.*, 2019). Although this data is specific for cerebellar functions, it indicates that age-related brain functions are not always linear.

The consequences of increased dentate-gyrus excitability in early aging have not been previously explored. More extensive research into late aging has revealed that senescence has an impact on LTP maintenance and synaptic plasticity. Aged rats have

deficits in both LTP induction and maintenance at the perforant path-granule cell synapse, which is linked to reduced spatial-memory performance (Barnes, 1979). Interestingly, intrinsic recordings of dentate gyrus granule neurons indicate that LTP is not affected between young (30 days old) and middle aged rodents (9 months old) but is significantly reduced in old rodents (18+ months old) (Barnes, Rao and Houston, 2000). It is possible that the "peak" of the bell curve was not identified in these experiments as rodent cohorts of 6 months of age were not assessed. In contrast, LTP magnitude in the CA1 has been shown to significantly increase between juvenile mice aged 10-15 weeks old and adult mice aged 26-38 weeks old (Diógenes *et al.*, 2011). The notion of non-linear changes is supported by other work where the maximum frequency potentiation of granule cells was not reduced between young and middle aged rats but was significantly reduced in aged rodents (Kerr *et al.*, 1991).

3.3.1.2 Age-dependent changes in dentate gyrus excitability are subregion specific

My data suggest an early-age-dependent increase in the excitability of both the infra- and the supra-pyramidal blades of the dentate gyrus in response to perforant pathway activation. However, I also demonstrate that the age-dependent responses are markedly higher in the supra-pyramidal dentate gyrus. Although age-related differences in subregional excitability have not been previously reported, functional and structural differences between the supra-pyramidal and infra-pyramidal blades have been demonstrated.

In particular, the immediate early genes *Arc* and *zif268*, which are involved in synapse plasticity regulation and are induced in response to neural activation during information processing (Jones *et al.*, 2001; Bramham *et al.*, 2008; Epstein and Finkbeiner, 2018) show higher expression in supra-pyramidal granule cells during spatial learning tasks (Schmidt, Marrone and Markus, 2012). Furthermore, pattern separation, a process that minimises the overlap between patterns of neuronal activity representing similar experiences (Madar, Ewell and Jones, 2019), is thought to occur almost exclusively in the supra-pyramidal region (Chawla *et al.*, 2005; Satvat *et al.*, 2011); the supra-pyramidal region receives greater medial perforant pathway input associated with spatial-navigation (Fyhn *et al.*, 2004); Arc expression is selectively reduced in the supra-pyramidal dentate gyrus after spatial exploration in aged rats (Marrone *et al.*, 2012); and

reduction in memory performance in aged mice is associated with impaired reactivation of neuronal assemblies in the supra-pyramidal blade and not the infra-pyramidal blade (Karaca *et al.*, 2021).

Cellular morphology is also different between the subregions of the dentate gyrus. For example, supra-pyramidal granule cells have greater dendritic length and spine densities (Desmond and Levy, 1982; Desmond and Levy, 1985; Claiborne, Amaral and Cowan, 1990), which are associated with are larger number of synaptic contacts (Sorra and Harris, 2000). Also, approximately 70% of granule cell dendrite length process perforant path signals (Raisman, Cowan and Powell, 1965; Fifková, 1975); as dendritic spines represent potential sites of post-synaptic input, larger granule spine length and number is translated to a larger number of excitatory synapses from the perforant path (Sorra and Harris, 2000). This is supported by the supra-pyramidal subregion expressing greater c-Fos staining than the infra-pyramidal subregion, highlighting the supra-pyramidal blade is linked to greater neuronal activity (Chawla *et al.*, 2013).

Together, these findings suggest that aging affects the supra-pyramidal region to a greater extent than the infra-pyramidal region, supporting my findings in this chapter. In a similar manner, these findings suggest that the infra-pyramidal subregion of the dentate gyrus is more resilient to age-related alterations. It is possible that this resilience manifests due to higher levels of cell proliferation and neurogenesis observed in the infra-pyramidal blade in rats after performing a spatial navigation task (Snyder *et al.*, 2009).

3.3.2 Early aging affects intrinsic membrane properties of granule cell neurons

3.3.2.1 EPSPs in granule neurons are not affected during early aging

By measuring the intrinsic properties of granule cells from the supra-pyramidal and infra-pyramidal dentate gyrus, I found that granule neuron EPSP elicited by perforant pathway stimulation did not differ between juvenile and adult mice. This suggests that the increased population excitability seen in early aging is not generated by an increase in postsynaptic responses in granule neurons. My observations differ from those found during late aging, which show that perforant path elicited granule cell EPSPs are reduced in late aging due to changes in synaptic number (Barnes, 1979) and synaptic potential (Barnes and McNaughton, 1980; Foster *et al.*, 1991). However, there are no reports of these synaptic parameters changing with early aging, so it is possible that other intrinsic

properties could be influencing cellular activity. It is important to note, that the significant changes in resting membrane potential will have an affect on the EPSP amplitude based on their closeness to the reversal potential.

Although granule neurons comprise a large percentage of the dentate gyrus granule cell layer, the dentate gyrus is populated by a diverse population of inhibitory interneurons, which provide inhibitory modalities to granule cells and can directly regulate granule cell excitability (Freund and Buzsaki, 1996) and the balance of excitation and inhibition is crucial for maintaining proficient memory performance during aging (Tran *et al.*, 2019). Down-regulation of inhibitory post-synaptic currents in the dentate gyrus is associated with a reduction in spatial learning and memory in rats (Tran, Gallagher and Kirkwood, 2018) and interestingly, $GABA_A$ receptor mediated tonic current is increased in early aging between rats 30-40 days old and 70-80 days old (Fleming, Wilson and Swartzwelder, 2007). These data indicate that dentate gyrus inhibitory currents also follow a “bell-shaped curve” with progressive aging, suggesting that changes in population excitability described in this chapter could be caused in part by inhibitory pyramidal neurons influencing granule cell activity. Genes associated with GABA-ergic inhibitory function are down regulated in the hippocampus during late aging in humans and primates (Loerch *et al.*, 2008), and a significant reduction in GABA-ergic inhibitory neurons are observed in the CA1 in rats (Stanley, Fadel and Mott, 2012; Vela *et al.*, 2003). Measuring the intrinsic inhibition properties of pyramidal neurons in the dentate gyrus and investigating cell number in early aging could help to provide more mechanistic insight into the findings presented in this chapter.

The most common interneuron in the dentate gyrus is the basket cell, which has a strong influence on granule cell action potential generation though a network of feedforward inhibition by the entorhinal cortex and feedback inhibition by mossy fibre collaterals (Hefft and Jonas, 2005; Feng *et al.*, 2021). The ratio of basket cells to granule cells is larger in the infra-pyramidal blade with an approximate 1:180 ratio respectively, versus a ratio of 1:100 in the supra-pyramidal blade (Seress and Pokorny, 1981). Whether differences in basket cell ratio between the subregions give rise to a difference in excitatory-inhibitory subregional balance is yet to be explored, however could be in part responsible for excitatory regional differences observed.

3.3.2.2 An increase in granule neuron membrane resistance and hyperpolarisation is observed in early aging

In this chapter, I demonstrate that input resistance and resting membrane potential in granule neurons from the infra-pyramidal blade are not altered during early aging. This finding is supported by previous work in the infra-pyramidal blade, which found that resting membrane potential and input resistance of granule neurons are not altered between young (30 days old), adult (9-10 months old) and old (25-27 months old) rats (Barnes, Rao and Houston, 2000).

In contrast, I found that granule neurons located in the supra-pyramidal blade of the dentate gyrus exhibit increased membrane resistance and hyperpolarised resting membrane potential. These data suggest, therefore, that early aging affects the intrinsic membrane properties of granule neurons in a subregion-specific manner. The reasons for these changes remain unknown.

A potential explanation for the alterations in intrinsic membrane properties of granule neurons centres around the permeability of ions. The neuronal membrane potential can be affected by the extracellular and intracellular concentrations of sodium (Na+), potassium (K+), and chloride (Cl–) ions. The altered changes in intrinsic membrane properties could occur due to changes in the membrane permeability of granule neurons. To account for the increased membrane resistance, the permeability of the cell membrane to ions must decrease. Because the resting membrane potential is hyperpolarised, the altered permeability must arise due to decreased Na+ permeability. (Decreased permeability of K+ or Cl– ions would lead to a depolarised membrane potential according to the Goldman equation.) NALCN channels are sodium leak channels that are responsible for resting membrane potential that controls neuronal excitability (Lu *et al.*, 2007), and to the best of my knowledge, there are no current reports of subregion- or age-dependent differences in the expression levels of NALCN. However, NALCN knockout mice have hippocampal neurons that are hyperpolarised by approximately 10 mV compared to wildtype mice (Lu *et al.*, 2007). Further investigation into NALCN channel properties or expression levels could help explain the changes in membrane resistance and resting membrane potential during early aging.

Additionally, non-activating persistent Na+ current, which arise via voltage-gated sodium channels (Stafstrom, Schwindt and Crill, 1982), is involved in mediating resting

membrane potential and is capable of amplifying a neurons response to synaptic input in hippocampal neurons (Stuart and Sakmann, 1995; Stuart, 1999; French *et al.*, 1990). Further investigating persistent sodium currents in my juvenile and adult mice cohorts could help explain the changes in resting membrane potential in early aging. This could be conducted by whole-cell voltage-clamp recordings of granule neurons in the subthreshold voltage range, and the sodium channel blocker tetrodotoxin (TTX) could be used to characterise the sodium currents (Jinno, Ishizuka and Kosaka, 2003).

Changes in intrinsic membrane potential could also occur via increased extracellular Cl- concentrations via voltage-gated chloride channels, (CLC channels) or decreased Na+ concentrations via NALCN channels. There are no current reports of age- or subregion-dependent differences in extracellular ion concentrations. However, reduction of CLC channels significantly reduces CA3 pyramidal cell number in early aging (1-3 month old mice versus 6-10 month old mice) (Cortez *et al.*, 2010). Further investigation into CLC channel properties or extracellular ion concentrations in the dentate gyrus could help to explain the changes of resting membrane potential detected in early aging.

To the best of my knowledge there are no previous reports showing that resting membrane resistance is affected by aging. However reduction of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels, significantly increases membrane resistance in dentate gyrus granule cells (Mishra and Narayanan, 2021). HCN channels are widely expressed in the hippocampus and play essential roles in modulating synaptic plasticity and learning and memory (Huang and Hsu, 2003; Lewis and Chetkovich, 2011; Nolan *et al.*, 2004) and are activated by membrane hyperpolarisation (Benarroch, 2013). These channels control the activities of hippocampal glutamatergic neurons and synaptic activity (Sinha and Narayanan, 2015; Robinson, 2003) and their expression levels in dentate gyrus granule neurons change in early aging (in 1 month vs 3 months old gerbils) (Lee, Park and Won, 2019). Investigation into HCN channel expression in my aging model may help to uncover some mechanistic insight behind the changes in granule cell neuron resistance in early aging.

3.3.3 Early aging does not affect the cortical-hippocampal gamma network

Increases in dentate gyrus excitability in early aging does not affect cortical-hippocampal gamma oscillations and the synchrony between the entorhinal cortex and CA3 at the

gamma frequency remained unaffected by early aging. This suggests that the changes in intrinsic granule neuron properties observed in early aging does not affect cortical-hippocampal network rhythms. Gamma oscillations are affected in late aging however, with both the power and frequency of gamma oscillations decreased in aged human subjects (50-88 years old) compared to younger subjects (20-48 years) (Murty *et al.*, 2020). Consistent with these observations, *in vitro* gamma power is strongly reduced in the CA3 of aged mice without changes in underlying synaptic connections (Vreugdenhil and Toescu, 2005; Lu *et al.*, 2011), yet no investigation has been made in early aging.

It is possible that changes in dentate gyrus excitability and intrinsic granule cell properties are affecting gamma oscillations more locally in the dentate gyrus, however very few experiments examine the effects of aging on dentate gyrus gamma oscillations. In the awake rat it appears that the dentate gyrus is the dominant source of gamma frequency activity in the hippocampal formation, with oscillations exhibiting a higher power and frequency than in all other regions (Bragin *et al.*, 1995). This subregions oscillatory activity with aging could be an important factor for exploration, and it is possible to record gamma oscillations from the dentate gyrus *in vitro* (Towers *et al.*, 2002),

Theta oscillations (4-12 Hz) are also present in the hippocampus, identified in the dentate gyrus and CA3 (Buzsaki, 2005), and have been linked to spatial information processing in hippocampus (Gupta *et al.*, 2012) and the formation of episodic memories (Lega, Jacobs and Kahana, 2012). It has been hypothesised that theta and gamma oscillations may be important for separating encoding and retrieval states in the hippocampus (Colgin *et al.*, 2009; Cutsuridis, Cobb and Graham, 2010) and similarly, phase-amplitude coupling between theta and gamma oscillations increase during successful encoding (Lega *et al.*, 2016; Mormann *et al.*, 2005). Theta oscillations in the hippocampus are affected in early and late aging. Theta power is reduced in the CA1 during running speed tasks in older adults (9 months old) versus young adults (4 months old) (Huxter, Miranda and Dias, 2012), and baseline theta power and running task-mediated theta power is reduced in late aging (Kuo *et al.*, 2010; Jacobson *et al.*, 2013). These results highlight that theta oscillations play an important role in hippocampal-mediated activity and are affected in early aging, therefore exploration into theta oscillations in the hippocampus in my early aging model could help to investigate whether hippocampal network activity is affected.

3.4 Future work

As changes in population EPSPs in the dentate gyrus have been associated with a reduction in synapse number and function, investigating these parameters in my juvenile and adult age cohorts could provide further mechanistic insight into fEPSP increase in early aging. Synaptic plasticity could be investigated by measuring LTP in dentate gyrus granule neurons elicited by theta burst stimulation in both age cohorts. Synaptic number investigation would be conducted by performing immunohistochemical staining of hippocampal slices staining for pre-synaptic and post-synaptic markers, such as synaptophysin and PSD-95, respectively. This technique would also allow for subregional investigation of synaptic number, indicating whether differences in synapse number between the supra-pyramidal and infra-pyramidal could be responsible for differences in their excitability.

Although granule neurons are the major cell of the dentate gyrus, inhibitory pyramidal neurons affect granule neuron excitatory activity, and inhibitory-excitation balance is important for proficient memory performance during aging (Tran *et al.*, 2019). Measuring pyramidal cell intrinsic properties and quantifying their number in the dentate gyrus could help to indicate whether inhibitory neurons play a role in fEPSPs changes during early aging.

I show that granule neuron hyperpolarisation occurs in supra-pyramidal granule neurons with aging, although the underlying mechanism for this change is unknown. Changes in extracellular chloride or intracellular sodium can affect membrane potential properties. Measuring the extracellular concentration of chloride ions in the dentate gyrus could be conducted to explore whether these ions are specifically involved in membrane potential changes. This could be conducted in hippocampal slices *in vitro*, measuring extracellular chloride concentration in both dentate gyrus subregions using ion-sensitive electrodes. Additionally, changes in resting membrane potential could also be attributed to altered expression of NALCN channels or changes in persistent sodium current. Voltage-clamp recordings could be used to investigate NALCN channel properties in dentate gyrus granule neurons and persistent sodium currents.

Dentate gyrus excitability in early aging does not affect cortical-hippocampal gamma oscillation power, frequency, or synchrony. These parameters were measured from the

entorhinal cortex and CA3, however early aging could be affecting gamma oscillations solely in the dentate gyrus. Furthermore, theta oscillations and gamma-theta coupling are important in episodic memory formation and are affected in early aging. Therefore, in order to conclude that age-related increases in dentate gyrus excitability do not affect hippocampal network oscillations, cortical-hippocampal theta and dentate gyrus gamma and theta oscillations should also be examined.

3.5 Summary

The results in this chapter show that hippocampal excitability increases in early aging. These results are different to late aging rodent studies, which illustrate that hippocampal excitability decreases between young versus middle-aged rodents and young versus old rodents. Together, these data suggest that the excitability of the dentate gyrus may increase as rodents move into adulthood, but then decrease as the rodent transitions to an older age. I also demonstrate that the age-dependent responses are markedly higher in the supra-pyramidal dentate gyrus.

I found that granule neurons located in the supra-pyramidal subregion of the dentate gyrus exhibit increased membrane resistance and hyperpolarised resting membrane potential, whereas no differences were observed in granule neurons in the infra-pyramidal subregion. These data suggest that early aging affects the intrinsic membrane properties of granule neurons in a subregion-specific manner. The reasons for these changes remain unknown.

Increases in dentate gyrus excitability in early aging does not affect cortical-hippocampal gamma oscillations - the synchrony between the entorhinal cortex and CA3, and the amplitude at the gamma frequency remained unaffected. This suggests that the changes in intrinsic granule neuron properties observed in early aging does not affect cortical-hippocampal network rhythms.

Future work could involve investigating the mechanisms underlying the changes in resting membrane potential and resistance during early aging. This could involve measuring NALCN channel properties and persistent sodium currents using voltage-clamp techniques or measuring extracellular chloride concentrations using ion sensitive electrodes in juvenile and adult mice.

Chapter 4 – CCL2 modulates hippocampal excitability

4.1 Introduction

The innate immune system is the first line of defence that acts to remove foreign pathogens and maintain homeostasis (Beutler, 2004; Paludan *et al.*, 2021). Increasing evidence suggests that pro-inflammatory cytokines and chemokines are elevated during normal aging (Brubaker, Palmer and Kovacs, 2011). It is thought that this may impair our ability to mount efficient immune responses to encountered pathogens (Bruunsgaard *et al.*, 1999; Shaw and Nunnari, 2002), and contribute to various immune-related pathologies (McGeer and McGeer, 2004).

The brain has its own distinct innate neural-immune system which helps to maintain a healthy cerebral environment (Kielian, 2014). However, components of the peripheral immune system can also cross the blood-brain barrier to contribute to neuroinflammatory events (Gutierrez, Banks and Kastin, 1993). The brain also shows heightened inflammation with age partly due to the dysregulation of the innate neural immune system (Morales *et al.*, 2014), although it is not clear whether this increase is produced from the brain's innate immune cells, or from systemic inflammation. The brain is acutely sensitive to inflammatory molecules and the disrupted balance of anti- and pro-inflammatory mediators with aging greatly affects neuronal function and behaviour (Goshen and Yirmiya, 2009) and pro-inflammatory mediator increase in the hippocampus has been shown to impair hippocampal-dependent learning and spatial memory tasks (Gibertini *et al.*, 1995; Barrientos *et al.*, 2002). In this way, inflammation in brain tissue plays a significant role in the pathogenesis of diseases related to cognitive function and memory (Morales *et al.*, 2014; Hirsch, Vyas and Hunot, 2012).

CCL2 is a major pro-inflammatory chemokine that increases in normal aging and can cross the blood brain barrier and exert its pro-inflammatory effects in the brain through binding to its cognate receptor CCR2 (Pinke *et al.*, 2013; Deshmane *et al.*, 2009). CCL2 and CCR2 are constitutively expressed in CNS neurons and are detectable in brain regions such as the hypothalamus, substantia nigra, cerebellum and hippocampus (Banisadr *et al.*, 2005b; Gosselin *et al.*, 2005). Interestingly, CCL2 has been shown to directly enhance neuronal excitability and synaptic transmission in the cornu ammonis in rats (Zhou *et al.*, 2011), indicating that the chemokine plays a larger role in brain regulation than simply regulating immune function. It has also been described to function

as a neuromodulator in the central nervous system as its expression is co-localised with neurotransmitters (Jung *et al.*, 2008; Melik-Parsadaniantz and Rostene, 2008).

Previous work suggests that CCL2 may play an important role in mediating cognitive deficits in late aging. Indeed, CCL2 expression levels are associated with a faster rate of cognitive decline (Westin *et al.*, 2012) and is thought to contribute to the pathophysiology of neurodegenerative diseases including Alzheimer's disease and multiple sclerosis (Mahad and Ransohoff, 2003; Ishizuka *et al.*, 1997; Bettcher *et al.*, 2016). The role for CCL2 in early aging, however, is not well understood.

CCL2 expression levels increase in an age-dependent manner in mice (Yousefzadeh *et al.*, 2018). Increased serum CCL2 levels are also found in human subjects aged between 30-50 years of age, which does not impact overall immune functionality (Seidler *et al.*, 2010). It has yet to be determined, however, whether CCL2 affects excitability in early aging. The focus of this chapter, therefore, is to investigate whether CCL2 affects hippocampal excitability in an age-dependent manner. I hypothesised that CCL2 would increase dentate gyrus excitability as previously shown in the CA1 (Zhou *et al.*, 2011). In addition, as age-dependent increases in peripheral CCL2 have been observed in mice (Yousefzadeh *et al.*, 2018) and as CCL2 is expressed in the hippocampus and has an affect cognitive decline (Westin *et al.*, 2012; Banisadr *et al.*, 2005), I hypothesised that CCL2 would be, in part, responsible for mediating age-dependent increases in hippocampal excitability.

4.2 Results

4.2.1 CCL2 increases excitability in the dentate gyrus

As CCL2 has been described to affect hippocampal function through enhancing neuronal excitability and synaptic transmission in the cornu ammonis in rats (Zhou *et al.*, 2011), and is increased in serum in middle-age (Seidler *et al.*, 2010; Yousefzadeh *et al.*, 2018), I investigated whether CCL2 could be responsible for mediating the increase in dentate excitability upon perforant pathway stimulation in early aging. This was conducted using the same experimental procedure described in 3.2.1, however dentate gyrus excitability generated through perforant pathway stimulation was measured before and after the addition of CCL2 to investigate whether the chemokine has direct effects on dentate gyrus excitability. *In vitro* experiments are useful tools to explore the effects of singular

immune components on cellular activity due to lack of influence from the circulatory system. Hippocampal slices were prepared from juvenile (9-15 weeks old) and adult (25-35 weeks old) wild-type mice. Once slices had been left to acclimatise to the *in vitro* conditions for 1 hour, control recordings were taken by electrically stimulating the entorhinal cortex layer II from 5V to 100V and measuring subsequent field EPSPs (fEPSPs) in the supra- and infra-pyramidal dentate gyrus, and a stimulation response curve was generated. Recombinant rat CCL2 was added to circulating ACSF for a bath concentration of 7 nM and slices were left to incubate for 1 hour, before a second stimulus response curve was generated.

The addition of CCL2 significantly increased dentate gyrus fEPSPs in the supra-pyramidal and infra-pyramidal subregions in juvenile mice (supra-pyramidal dentate gyrus: control, 0.50 ± 0.02 vs +CCL2 1.43 ± 0.23, P=0.0001. Infra-pyramidal dentate gyrus: control, 0.51 ± 0.02 vs +CCL2, 1.90 ± 0.34, P<0.0001). In adult mice, basal levels of excitability are greater than juvenile mice (as observed in 3.2.1), and CCL2 incubation still increases excitability further (supra-pyramidal dentate gyrus: control, 0.5 ± 0.01 vs +CCL2, 1.10 ± 0.15, P=0.030. Infrapyramidal dentate gyrus: control, 0.48 ± 0.02 vs +CCL2, 1.03 ± 0.18, P=0.012), Fig 4.1 C. However, this CCL2-meidated excitability increase has a smaller amplitude in adult mice compared to juveniles in the infra-pyramidal subregion (Juvenile mice +CCL2 1.90 ± 0.34, vs adult mice +CCL2 1.03 ± 0.18, P=0.0124, Fig 4.1 C). Plotted stimulus response curves showing the slope of fEPSPs as a function of stimulation intensity in juvenile and adult mice, with and without CCL2 treatment also highlight that CCL2 significantly increased dentate gyrus excitability (Fig 4.1 D). These data strongly indicate that CCL2 increases dentate gyrus excitability upon perforant pathway stimulation, however no significant age-dependent affect was observed. Although CCL2 directly effects hippocampal excitability, it is not exclusively mediating the age-dependent increase in hippocampal excitability reported in the previous chapter (Fig 3.1).

It is evident that CCL2 increases excitability in the dentate gyrus during perforant pathway stimulation as shown in figure 4.1, however it is not yet clear whether this effect is exclusively caused by CCL2 or whether other pro-inflammatory chemokines would have the same effect. CCL5 is another pro-inflammatory chemokine that is upregulated in the blood in normal aging (Pulsatelli *et al.*, 2000), has been associated with

accelerating the development of neurodegenerative disorders such as Alzheimer's Disease (Li and Zhu, 2019) and *in situ* hybridisation studies indicate that CCL5 mRNA is expressed by neurons in the hippocampus. As CCL5 and CCL2 share similar age-dependent characteristics, I next investigated whether CCL5 exerts the same effects on dentate gyrus excitability as CCL2.

The experimental setup was the same as described in (Fig 4.1), however after control recordings were taken, recombinant murine CCL5 was added to circulating ACSF for a bath concentration of 1.28 nM. The CCL5 and CCL2 bath concentrations used here differ slightly but were chosen on the basis that these concentrations of CCL2 and CCL5 are sufficient to induce 95% chemotaxis of T-cell populations.

CCL5 addition significantly increased infra-pyramidal dentate gyrus fEPSPs in juvenile wild-type mice (control, 0.51 ± 0.02 vs +CCL5, 1.22 ± 0.28, p=0.014). However, this increase in excitability was not as profound as the effects observed after CCL2 addition (Fig 4.1), nor did CCL5 incubation result in any significant excitability changes in the other experimental groups (Fig 4.2). These data indicate, that although CCL5 does affect hippocampal excitability in a region-specific manner, CCL2 has a much more profound effect on the subregion's activity.

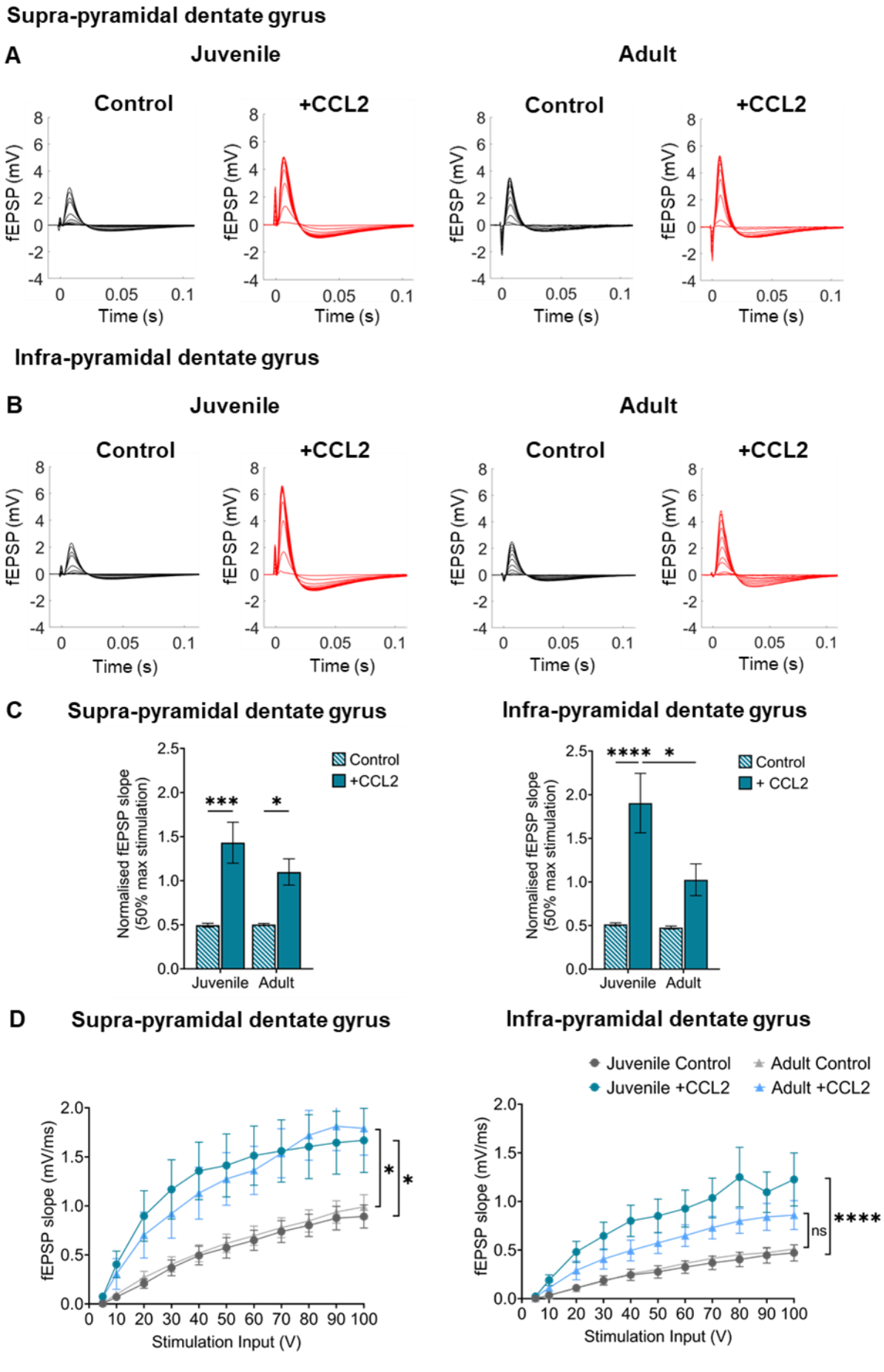

Supra-pyramidal dentate gyrus
A
Juvenile
Adult
Control
+CCL2
fEPSP (mV)
Time (s)
Infra-pyramidal dentate gyrus
B
C
Supra-pyramidal dentate gyrus
Infra-pyramidal dentate gyrus
Normalised fEPSP slope (50% max stimulation)
Control
+CCL2
+ CCL2
Juvenile
Adult
***
*
****
D
fEPSP slope (mV/ms)
Stimulation Input (V)
Juvenile Control
Adult Control
Juvenile +CCL2
Adult +CCL2
ns

Figure 4.1 CCL2 increases excitability of the dentate gyrus in juvenile and adult mice. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. The perforant path was activated by electrical stimulation of the entorhinal cortex layer II. Control recordings were taken from slices incubated with ACSF and again after slices had been treated with 7 nM CCL2 for 1 hour. **(A–B)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal (A) or infra-pyramidal blade (B) of the dentate gyrus in juvenile or adult mice from control slices or with 7nM CCL2. The entorhinal cortex was stimulated at time 0. fEPSPs represent the average response of 10 electrical stimulations for each intensity (5V–100V). **(C)** Grouped data of normalised fEPSPs at 50% maximum stimulation intensity recorded from the supra-pyramidal and infra-pyramidal dentate gyrus in juvenile or adult mice, with and without CCL2 treatment. **(D)** Stimulus response curves showing the slope of fEPSPs as a function of stimulation intensity in juvenile or adult mice, with and without CCL2 treatment, recorded from the supra-pyramidal and infra-pyramidal dentate gyrus. For (D), significance level is described at 100V stimulation input only. Bars represent mean ± SEM. * P ≤ 0.05, *** P ≤ 0.001, **** P ≤0.0001, two-way ANOVA with Tukey multiple comparison tests. N=11 juvenile mice and N=10 adult mice for each group (where N represents recordings from 1 hippocampal slice per animal).

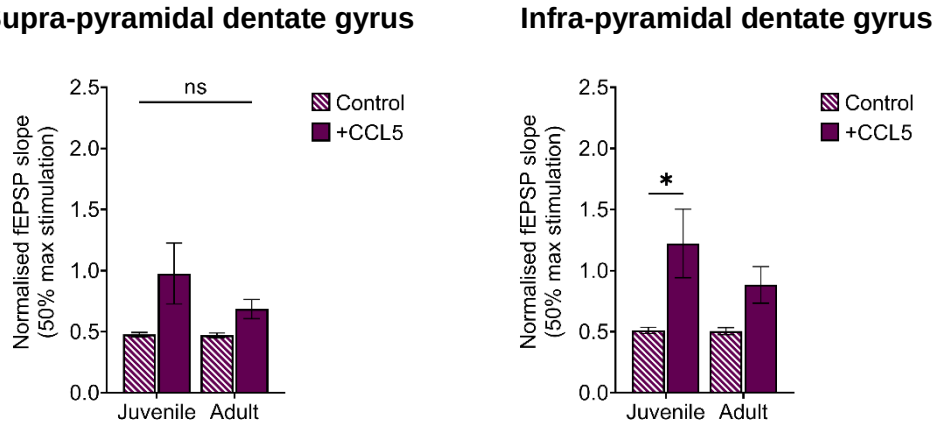


Figure 4.2 CCL5 increases dentate gyrus excitability in the infra-pyramidal dentate gyrus in juvenile mice. Hippocampal slices were prepared from juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice and the perforant path was activated by electrical stimulation. Control recordings were taken from slices incubated with ACSF, and then again after incubation with 1.28 nM CCL5 for 1 hour. Results are presented as normalised field-EPSPs (fEPSPs) at 50% maximum stimulation intensity recorded from the supra-pyramidal and infra-pyramidal dentate gyrus in juvenile or adult mice, with and without CCL5 treatment. Bars represent mean ± SEM. * P ≤ 0.05, two-way ANOVA with Tukey multiple comparison tests. N=8 mice for each group (where N represents recordings from 1 hippocampal slice per animal).

4.2.2 Hippocampal activity in slices is unaffected by time spent in *in vitro* conditions

The results in this chapter provide strong evidence that CCL2 affects hippocampal excitability, however it is good practise to ensure that the experimental design setup is not influencing slice physiology. It is possible that hippocampal slices take longer than the initial one hour to fully acclimatise to the *in vitro* experimental conditions, and therefore the length of time spent in these conditions could affect output recordings. I

conducted a control experiment to test whether leaving hippocampal slices in circulating ACSF affected hippocampal excitability upon perforant pathway stimulation. Wild-type juvenile hippocampal slices were left to acclimatise to the *in vitro* conditions for one hour before a stimulus response curve was measured in the supra-pyramidal dentate gyrus as described in 4.1.1. Slices were then left for one hour in circulating ACSF before a second stimulus response curve was generated.

Bathing slices in ACSF for one hour does not affect fEPSPs upon perforant pathway stimulation (Fig 4.3). This highlights that time spent in *in vitro* conditions does not impact hippocampal measurements, and hence the increase in hippocampal excitability seen with CCL2 (Fig 4.1) can be attributed to the biological actions of the chemokine.

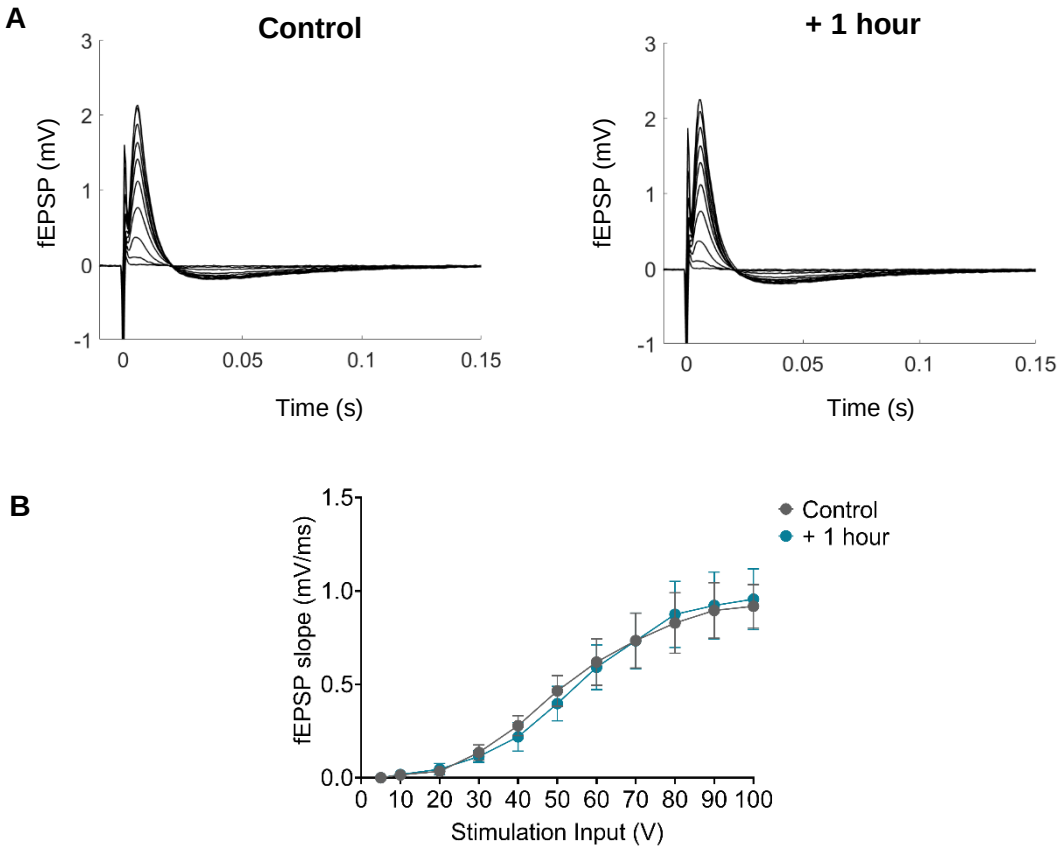


Figure 4.3 Bathing slices for 1 hour in ACSF does not affect field EPSPs. Hippocampal slices were prepared from juvenile (9-15 weeks of age) wild-type mice, and the perforant pathway was activated by electrical stimulation of the entorhinal cortex layer II. Control recordings were taken from slices incubated with ACSF before slices were left for 1 hour, and a second recording was taken. **(A)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal blade of the dentate gyrus in juvenile mice. The entorhinal cortex was stimulated at time 0. fEPSPs represent the average response of 10 electrical stimulations (1 Hz) for each intensity (5V–100V). **(B)** Stimulus response curves showing the slope of fEPSPs as a function of stimulation intensity for both control recordings. Bars represent mean ± SEM, two-way ANOVA with Sidak multiple comparison tests. N=5 mice for both groups (where N represents recordings from 1 hippocampal slice per animal).

4.2.3 Dimethyl sulfoxide increases hippocampal excitability

Most treatment compounds used in this thesis are reconstituted from a powdered form in double distilled water, which has no effect on brain slice behaviour *in vitro,* as water is the main compound in ACSF. However, the CCR2 antagonist and CCR3 antagonist compounds are reconstituted in dimethyl sulfoxide (DMSO), a polar aprotic solvent. Once administered to circulating ACSF, the concentration of DMSO in these experiments is very small, approximately 0.01% (v/v). However, evidence indicates that DMSO added to ACSF at a concentration of 0.05% (v/v), a concentration generally considered innocuous, alters intrinsic excitability properties of hippocampal CA1 pyramidal neurons (Tamagnini *et al.*, 2014). Therefore, I next examined whether DMSO at a concentration of 0.01% (v/v) influenced dentate gyrus neuronal excitability.

The experiment was conducted in a similar manner to experiments described in sections 4.2.1 and 4.2.3. Hippocampal slices were prepared from wild-type juvenile mice, and fEPSPs were measured from the supra-pyramidal and infra-pyramidal dentate gyrus during perforant pathway stimulation. A control stimulus response curve was taken from slices bathing in circulating ACSF, before treating slices with 0.01% (v/v) DMSO for one hour, and a second stimulus response curve taken.

Treatment with 0.01% (v/v) DMSO significantly increased dentate gyrus excitability in the supra-pyramidal and infra-pyramidal dentate gyrus (Fig. 4.4 C) in mice. These data indicate, that even at very low concentrations, DMSO can actively influence cellular activity, particularly in the hippocampal subregion as described here and by Tamagnini *et al*., (2014). It is therefore critical, especially with experiments measuring subtle changes in neuronal activity, that experiments are designed to account for DMSO's influence on neuronal tissue. Therefore, for experiments that required the application of compounds made up in DMSO, I ensured that control recordings were taken from slices pre-incubated in the DMSO compound to account for any associated activity changes.

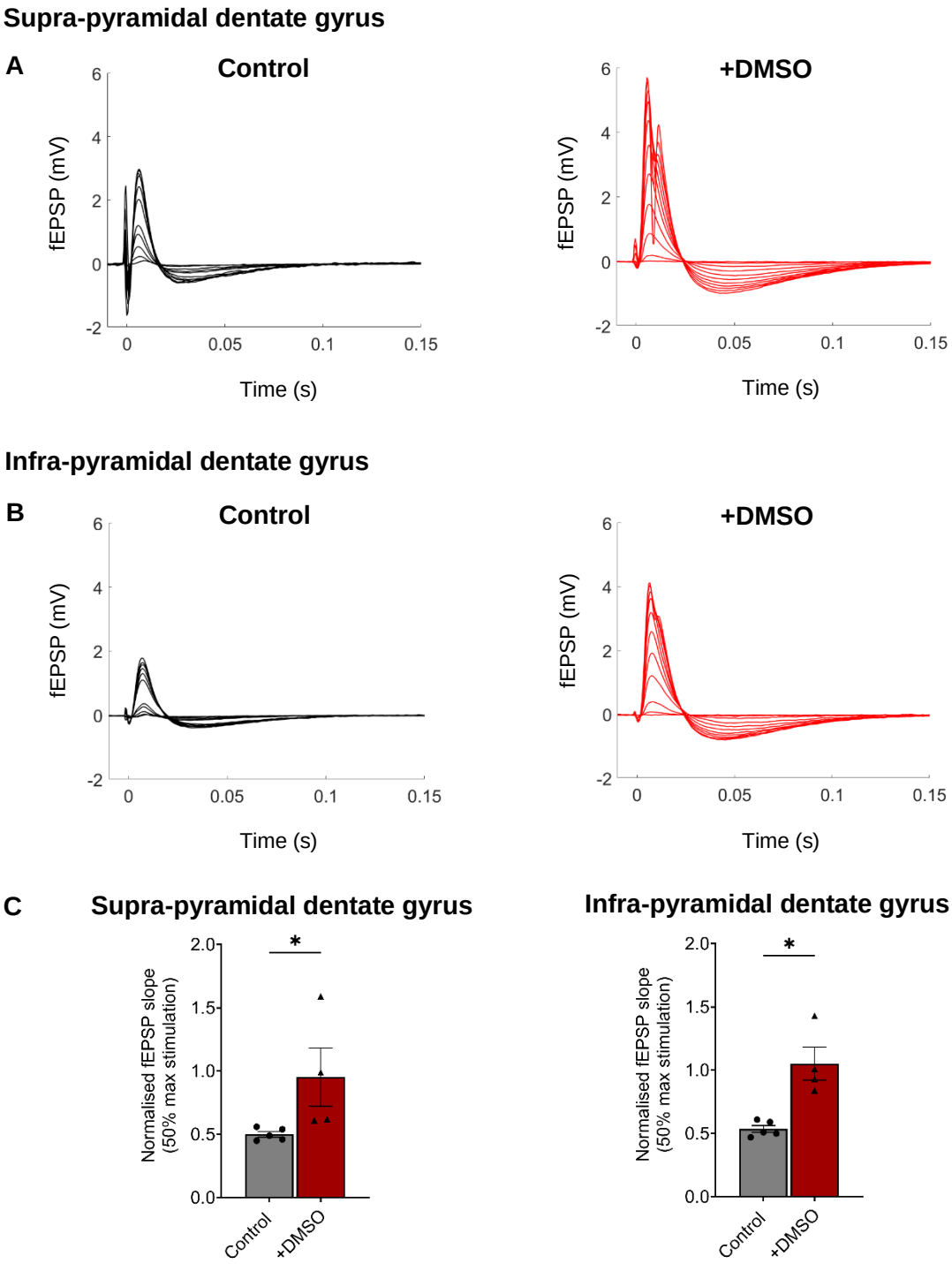


Figure 4.4 DMSO increases excitability of the dentate gyrus. Hippocampal slices were prepared from wild-type mice and the perforant path was activated by electrical stimulation of the entorhinal cortex. Control recordings were taken from slices incubated with circulating ACSF before slices were incubated with 0.01% (v/v) DMSO, and a second recording taken. **(A-B)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal blade and infra-pyramidal blade of the dentate gyrus before and after DMSO addition. fEPSPs represent the average response of 10 electrical stimulations (1 Hz) for each intensity (5V–100V). **(C)** Normalised fEPSPs at 50% maximum stimulation intensity recorded from the supra-pyramidal and infra-pyramidal dentate gyrus with and without DMSO treatment. Bars represent mean ± SEM. * $P \leq 0.05$, Mann-Whitney test. N=5 and N=4 for control and DMSO treated group respectively (where N represents recordings from 1 hippocampal slice per animal).

4.2.4 CCL2 exerts its effects on hippocampal excitability via CCR2

CCL2 has been shown to exert its biological effects by binding to its cognate receptor CCR2. Upon ligand binding, CCR2 induces monocytes to exit the bloodstream to act as tissue macrophages in response to inflammatory signalling (Deshmane *et al.*, 2009; Yousefzadeh *et al.*, 2018) and is one of the most prominent chemokine receptors associated with central nervous system inflammatory processes (Banisadr *et al.*, 2002; Sokolova *et al.*, 2009). As CCR2 is expressed in CNS neurons and has been shown to be localised to the hippocampus (Banisadr *et al.*, 2005b), I next examined whether CCL2 was exerting its effects on dentate gyrus excitability via CCR2.

This was conducted by treating hippocampal slices with a highly specific CCR2 antagonist (reconstituted in DMSO) before CCL2 addition, with the hypothesis that blocking CCL2-CCR2 ligand binding would significantly reduce CCL2's excitatory effect on dentate gyrus neurons. Hippocampal slices were prepared from either juvenile (9-15 weeks old) or adult (25-35 weeks old) wild-type mice, and after slices had acclimatised to the *in vitro* experimental conditions, the CCR2 receptor antagonist RS504393, which prevents binding of CCL2 to CCR2, was added to circulating ACSF for a bath concentration of 1µM. This concentration is described to inhibit 60% of CCL2 chemotaxis (Mirzadegan *et al.*, 2000). After slices had incubated with the antagonist for one hour, fEPSPs were measured from the supra-pyramidal and infra-pyramidal dentate gyrus during perforant pathway stimulation as previously described (3.2.1 and 4.2.1). Slices were then treated with 7nM of recombinant rat CCL2 for one hour before a second stimulus response curve was taken.

Pre-treating slices with a highly selective CCR2 antagonist, and thus blocking CCL2-CCR2 ligand binding, eliminated CCL2's effect on hippocampal excitability in both dentate gyrus subregions in juvenile and adult mice (Fig 4.5). These data indicate that CCL2-CCR2 receptor binding is a crucial mechanism in CCL2-mediated hippocampal excitability.

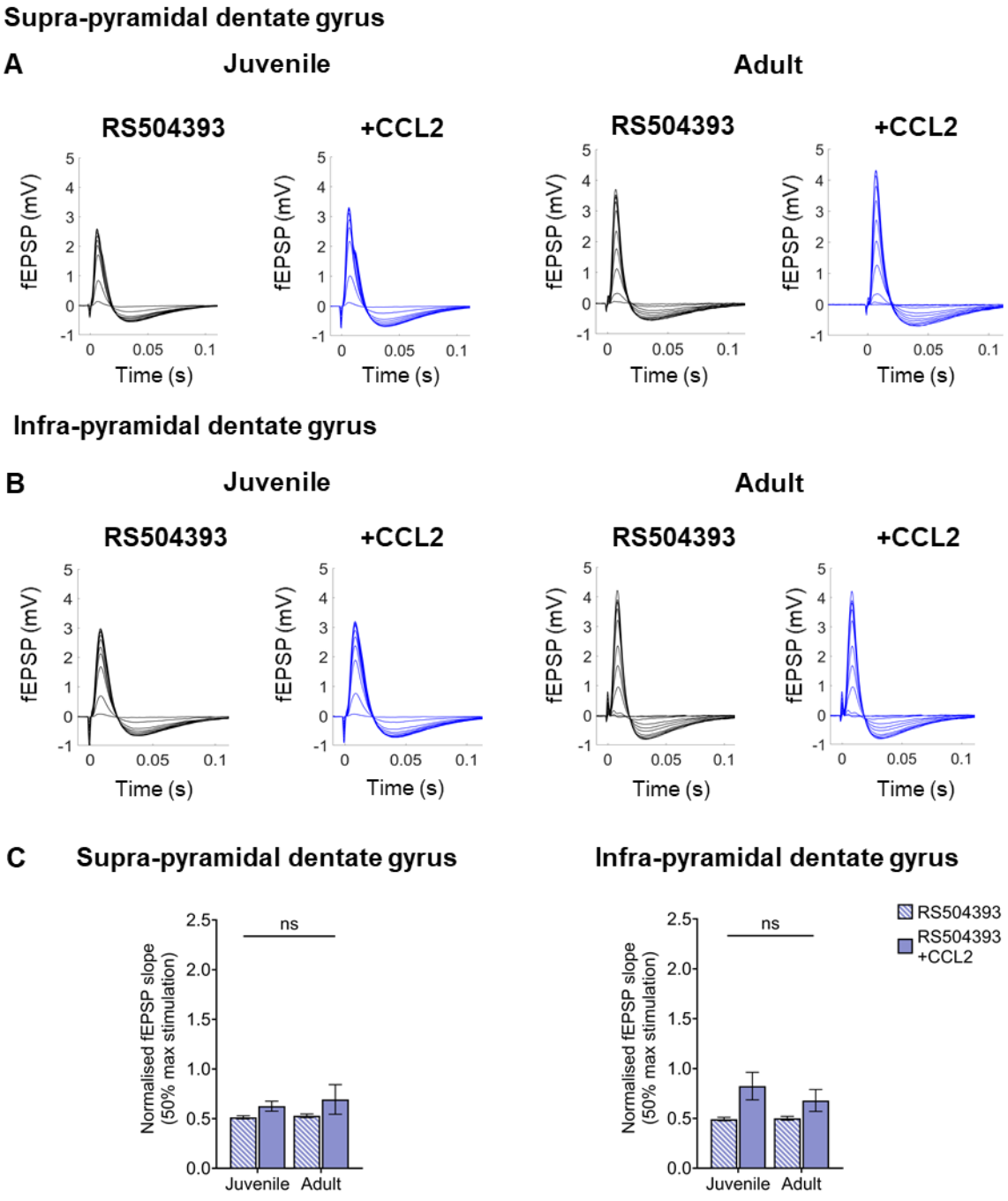


Figure 4.5 CCL2 increases hippocampal excitability via CCR2. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type mice. The perforant path was activated by electrical stimulation of the entorhinal cortex layer II. Recordings were taken after slices were incubated with 1µM of CCR2 antagonist RS504393 for 1 hour. Slices were then incubated with 7nM CCL2 for 1 hour and a second recording taken. **(A–B)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal (A) or infra-pyramidal blade (B) dentate gyrus in juvenile or adult mice, from slices pre-bathed with 1µM RS504393 or with 7nM CCL2. fEPSPs represent the average response of 10 electrical stimulations (1 Hz) for each intensity (5V–100V). **(C)** Grouped data showing fEPSPs at 50% maximum stimulation intensity. Data is normalised to slices pre-bathed in RS504393 treatment. Bars represent mean ± SEM, two-way ANOVA with Tukey multiple comparison tests. N=10 mice for each group (where N represents recordings from 1 hippocampal slice per animal).

4.2.5 CCL2 does not exert its effects on hippocampal excitability through CCR3

The experiments in this chapter indicate that CCL2 directly affects dentate gyrus activity (Fig 4.1) through binding to its cognate receptor CCR2, as treating slices with a selective CCR2 antagonist eliminated CCL2-mediated excitability (Fig 4.5). Although the CCR2 antagonist (RS504393) used in this experiment is claimed to be highly selective for CCR2, there is still a possibility that the compound could be non-specifically targeting other C-C chemokine receptors. If this is the case, the results observed in Fig 4.5 may not show reduced CCL2-mediated activity via CCR2 blocking, but instead a reduction in general hippocampal excitability through uniform down-regulation of several chemokine receptors.

To rule out non-specific binding of CCL2 to other chemokine receptors as a mechanism for the observed effects in figure 4.5, I investigated whether blocking a different proinflammatory chemokine receptor would affect CCL2-mediated hippocampal excitability. CCL2 is reported to function only when binding to CCR2 (Zhang, Patel and Pienta, 2010), so in theory the blocking of any other chemokine receptor should not alter CCL2's effect on hippocampal excitability. CCR3 is a pro-inflammatory chemokine receptor that is expressed in the hippocampus (van der Meer *et al.*, 2000), and is associated with age-related disease pathologies (Takeda *et al.*, 2009). I targeted this receptor by pre-treating hippocampal slices with 8 nM of the selective CCR3 antagonist SB328437 (reconstituted in DMSO) for 1 hour before a control stimulus response curve was recorded. Slices were then incubated with 7 nM CCL2 for 1 hour before a second stimulus response curve was taken.

CCL2 increased fEPSP responses in the dentate gyrus despite pre-incubation with the CCR3 antagonist SB328437 (Fig 4.6 B). These data highlight that presumed blocking of a non-specific pro-inflammatory chemokine receptor does not impede CCL2-mediated excitability. Therefore, the elimination of CCL2-mediated hippocampal excitability by RS504393 (Fig 4.5) is likely a result of specific inhibition of CCL2's cognate receptor, CCR2.

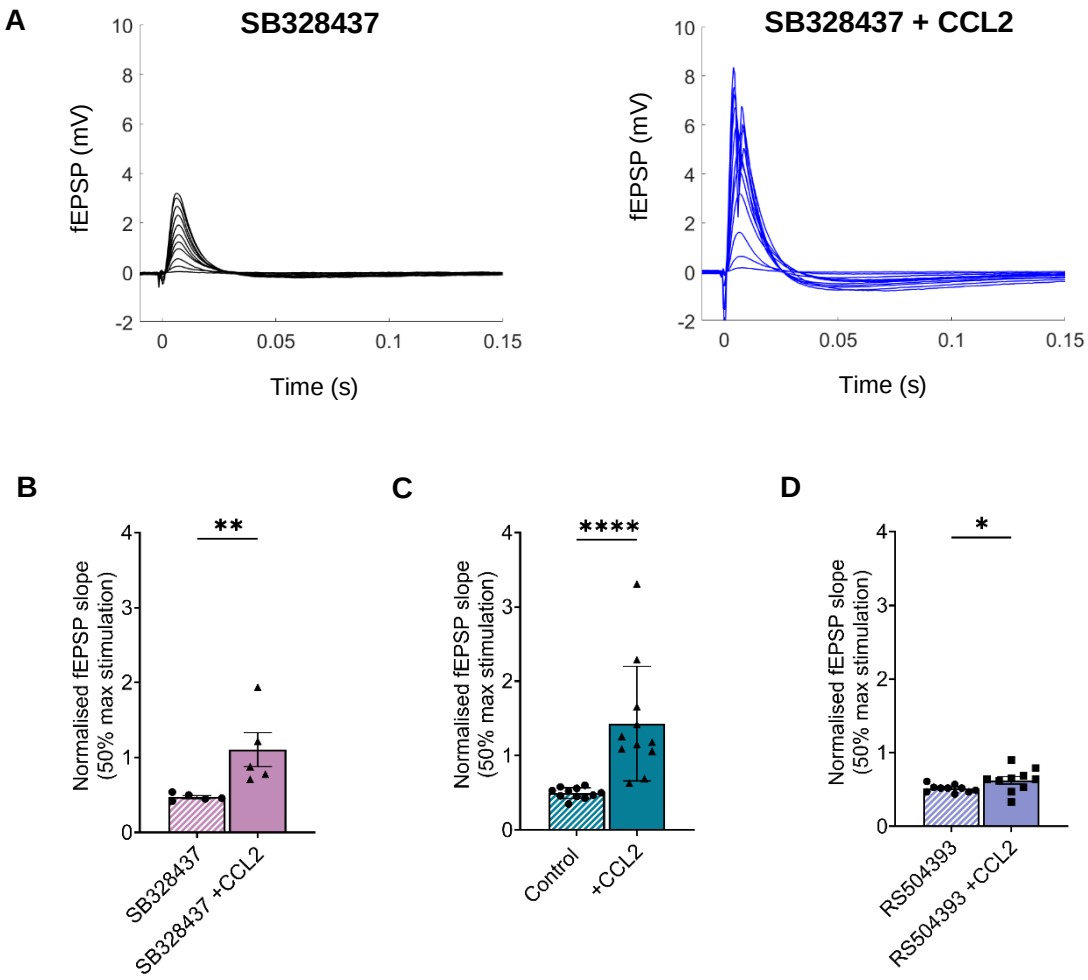


Figure 4.6 CCL2 does not exert its effects on hippocampal excitability via CCR3. Hippocampal slices were prepared from wild-type mice and the perforant path was activated by electrical stimulation of the entorhinal cortex. Control recordings were taken from slices pre-incubated with 8 nM circulating CCR3 antagonist SB32843, before incubation with 7 nM CCL2 for 1 hour and a second recording taken. **(A)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal blade of the dentate gyrus pre-bathed in SB328437 and incubated with CCL2. fEPSPs represent the average response of 10 electrical stimulations (1 Hz) for each intensity (5V–100V). (**B-D)** fEPSPs at 50% maximum stimulation intensity recorded from the supra-pyramidal dentate gyrus. (**B**) CCL2 increases fEPSPs after pre-incubation with a CCR3 antagonist. Data is normalised to slices pre-bathed in SB23843. N=5 mice for both groups. (**C-D**) Previous data shown for reference. CCL2 increases dentate gyrus excitability (C) and this response is eliminated when slices are treated with a CCR2 antagonist (D). Bars represent mean ± SEM (Mann-Whitney test), * P ≤ 0.05, ** P ≤ 0.01, **** P ≤ 0.0001.

4.2.6 CCL2 increases microglia branch length

Microglia are the major immune cell in the central nervous system and play an important role in innate immunity and inflammatory responses (Colonna and Butovsky, 2017). They are the primary sources of proinflammatory cytokines and are pivotal mediators of neuroinflammation (Liddelow, 2019). Microglia function is altered in aging and neurodegeneration, and increased levels of microglia have been associated with the pathology of age-related diseases (Streit, Mrak and Griffin, 2004). Microglia exist in resting or activated states depending on the inflammatory environment, (Ransohoff and Cardona, 2010) and in response to stimuli microglia have the ability to shift into different functional states that are accompanied by changes in cellular morphology (Cuadros and Navascués, 1998), proliferation (Gomez-Nicola and Perry, 2015; Gómez-Nicola *et al.*, 2013) and inflammatory cytokine and chemokine release (Kettenmann *et al.*, 2011). CCL2 is produced by microglia cells either in a constitutive manner, or in response to various stimuli, such as in response to infection or injury (Deshmane *et al.*, 2009). Under pathological conditions, CCL2 expression is increased in microglia (Minami and Satoh, 2003). CCL2 is also able to influence microglia activity and morphology, as neuron derived CCL2 can induce the production of new microglia and increase microglia proliferation (Hinojosa *et al.*, 2011)

As microglia are the major immune cells in the brain, and their function is altered in aging, I wanted to examine if microglia morphology and proliferation was affected in early aging. In addition, as microglia are involved in CCL2 production and CCL2-mediated immune responses, I examined whether hippocampal slices bathed in circulating CCL2 would influence microglia properties located around in the dentate gyrus. Microglial activation is accompanied by characteristic morphology changes in the arborisation of their fine projections and cellular shape.

Juvenile (9-15 weeks old) and adult (25-35 weeks old) hippocampal slices that were used for the *in vitro* electrophysiology experiment described in Fig 4.1, that were either incubated in circulating ACSF or 7nM CCL2 for 1 hour were collected, fixed, cryoprotected and re-sectioned. Immuno-fluorescence immunohistochemistry was conducted on re-sectioned brain slices staining for the microglia marker ionised calcium binding adapter 1 (Iba1), the mature neuronal marker Neu-N, and the nuclear stain Hoechst. The microglia staining was captured using confocal microscopy. Microglia

number was generated by counting the individual cells from confocal images at a x20 objective. Microglia morphological parameters of arborisation, maximum branch length and ramification index was calculated using Sholl analysis on confocal images captured at a x63 objective. A higher magnification was used to clearly identify microglia projections.

Microglia branch length was increased after CCL2 incubation in juvenile mice, Fig 4.7 C, however CCL2 incubation led to no other microglia morphology changes in juvenile (Fig 4.7) or adult mice (Fig 4.8).

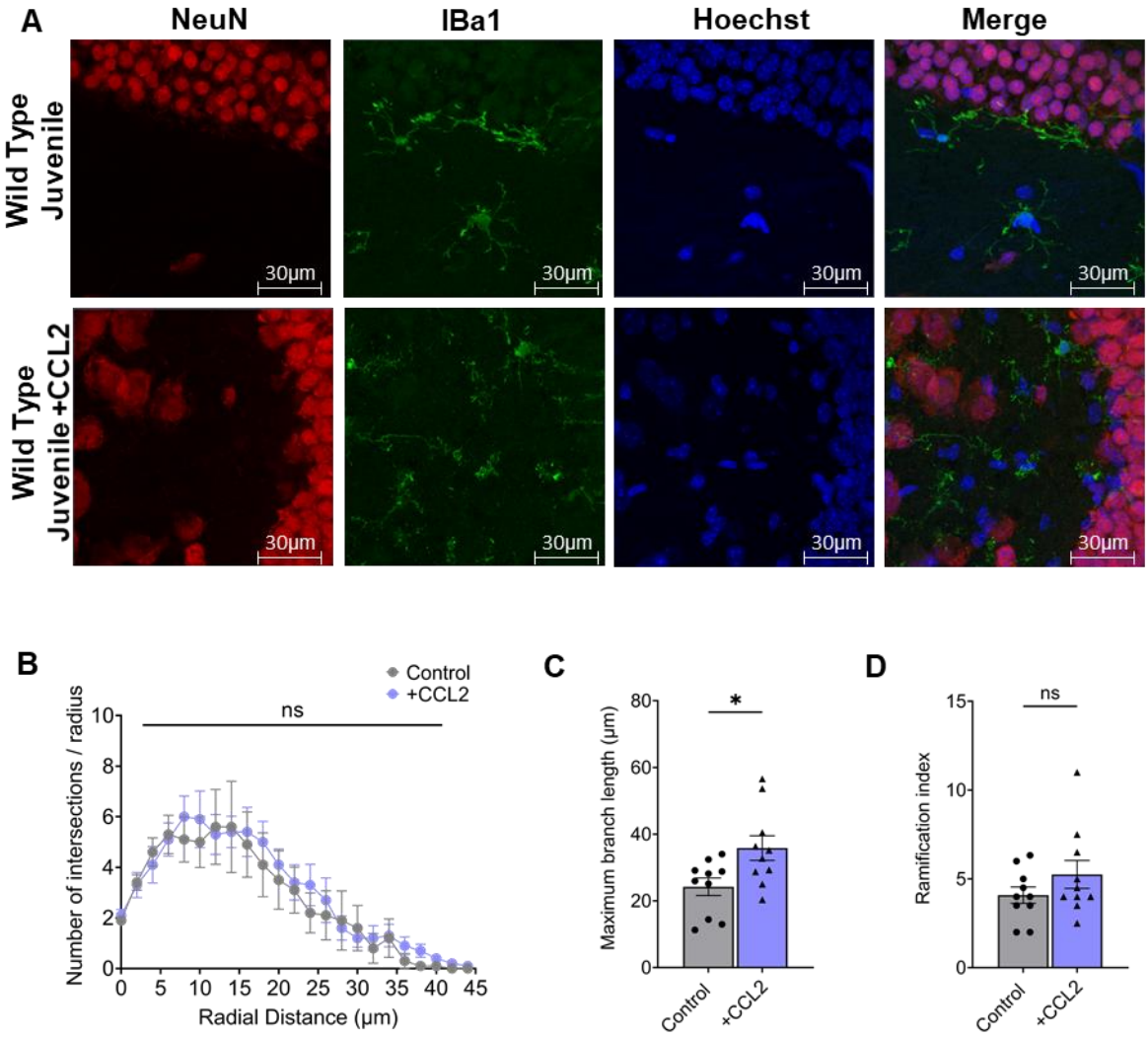


Figure 4.7 CCL2 increases dentate gyrus microglia branch length in juvenile mice. Wild-type hippocampal slices from juvenile (9-15 week old) mice used for electrophysiology were fixed in 4% PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Slices were collected that had been bathed in ACSF and in circulating 7nM CL2. Free-floating immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. The primary antibodies IBa1 microglia stain, NeuN mature neuronal stain and Hoechst nuclear stain were used. (**A**) Representative maximum intensity projection images of immuno-staining collected using a confocal microscope at a x63 objective. Single channel Iba1, NeuN and nuclear stain are presented along with a merge of all three channels for juvenile mice treated with control or CCL2. (**B-D**) Scholl analysis of captured images indicating the number of microglia branching intersections radial distance (B), maximum branch length (C) and ramification index (D). Bars represent mean ± SEM, * P ≤ 0.05, Mann-Whitney test. N=10 cells for both groups measured across hippocampal slices from 3 individual animals.

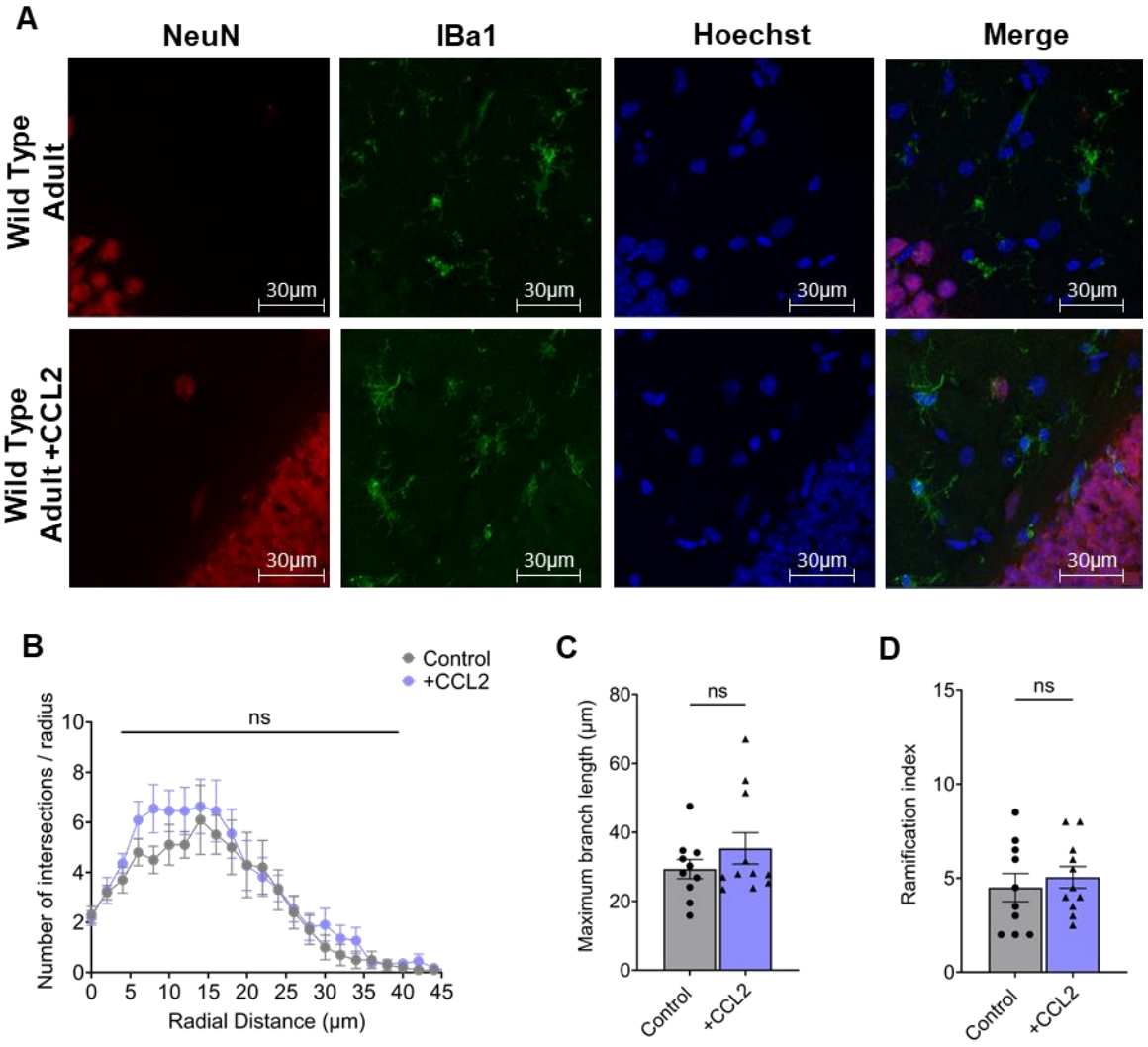


Figure 4.8 CCL2 has no effect on dentate gyrus microglia morphology in adult mice. Wild-type hippocampal slices from adult (25-35 weeks old) mice used for electrophysiology were collected, stored in PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Slices were collected that had been bathed in ACSF and in circulating 0.1µg/ml CCL2. Free-floating immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. The primary antibodies IBa1 microglia stain, NeuN mature neuronal stain and Hoechst nuclear stain were used. (**A**) Representative maximum intensity projection images of immuno-staining collected using a confocal microscope at a x63 objective. Single channel Iba1, NeuN and nuclear stain are presented along with a merge of all three channels for juvenile mice treated with control or CCL2. (**B-D**) Scholl analysis of captured images indicating the number of microglia branching intersections radial distance (B), maximum branch length (C) and ramification index (D). Bars represent mean ± SEM, Mann-Whitney test. N=11 cells for both groups measured across hippocampal slices from 3 individual animals.

Microglia undergo morphological changes in normal aging (Conde and Streit, 2006), however these studies compare young mice with very old (15-30 months of age), and there is sparse literature studying the effects of early aging. Using the same hippocampal slices gathered in the experiment described above (Fig 4.7 and 4.8), I measured microglia morphology by conducting Sholl analysis on juvenile and adult mouse hippocampal slices. These results show that early aging has no effect on microglia morphology in control slices or slices treated with CCL2 (Fig 4.9).

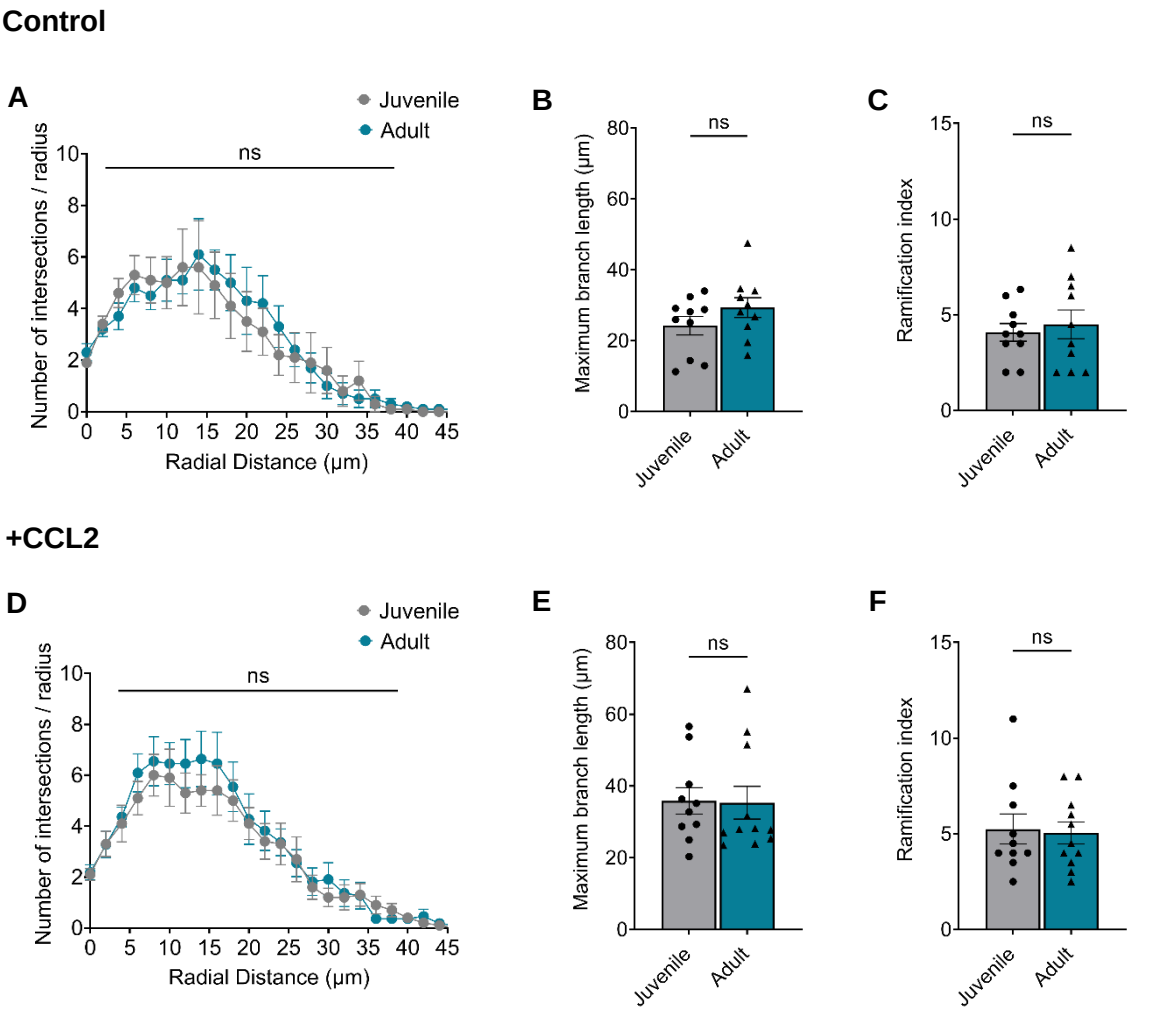


Figure 4.9 Dentate gyrus microglia morphology in unaffected by early aging. Wild-type hippocampal slices from juvenile (9-15 weeks old) and adult (25-35 weeks old) mice used for electrophysiology were collected, stored in PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Slices were collected that had been bathed in ACSF and in circulating 7nM CCL2. Immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. The primary antibodies IBa1 microglia stain, NeuN mature neuronal stain and Hoechst nuclear stain were used. (**A-F**) Scholl analysis of captured images indicating the number of microglia branching intersections radial distance in control (A) and CCL2 treated slices (D), maximum branch length for control (B) and CCL2 treatment (E), and ramification index for control (C) and CCL2 treatment (F). Bars represent mean ± SEM, Mann-Whitney test. N=11 cells for both groups measured across hippocampal slices from 3 individual animals.

There is a plethora of evidence indicating that microglia proliferate in aging, and that CCL2 signalling can also induce this effect (Hinojosa *et al.*, 2011). In order to measure cell proliferation, I used the same immuno-stained hippocampal sections used in the experiments described above, and re-imaged slices at a x20 objective to capture a larger area view of the dentate gyrus. I manually counted the number of Iba1 positive cells in the hilus and cortical region of the hippocampus in slices treated with control and 7nM CCL2. These results show that there is no significant difference in microglia cell number in the cortex or hilus between juvenile and adult mice, nor between slices treated with and without CCL2 (Fig 4.10), indicating that a 1 hour incubation with CCL2 does not result in significant microglia proliferation.

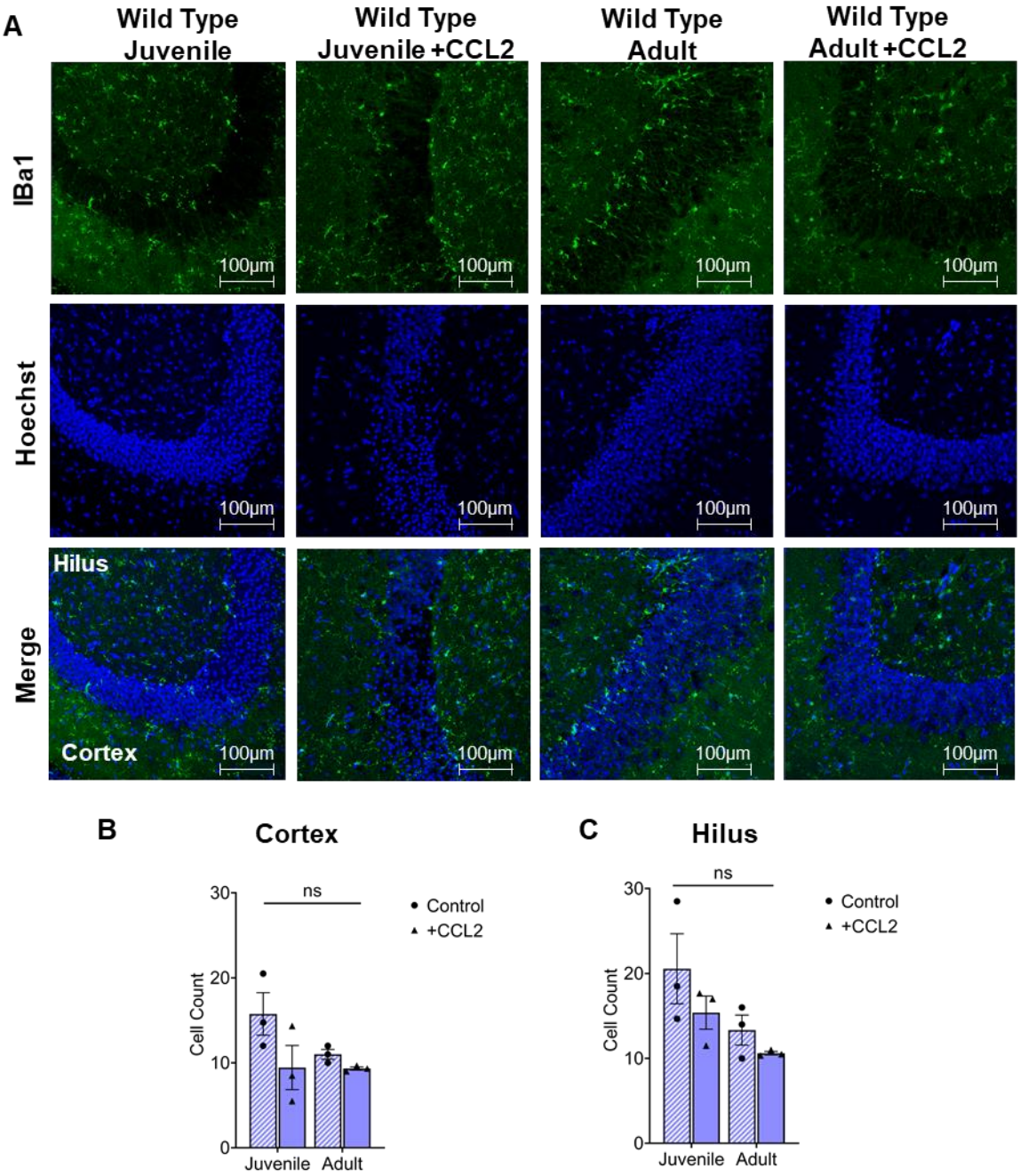


Figure 4.10 CCL2 has no effect on microglia number. Hippocampal slices from juvenile (9-15 weeks old) and adult (25-35 weeks old) mice used for electrophysiology were collected, stored in PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Slices were collected that had been bathed in ACSF and in circulating 0.1µg/ml CCL2. Free-floating immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. The primary antibodies IBa1 microglia stain and Hoechst nuclear stain were used. (**A**) Representative maximum intensity projection images of immuno-staining collected using a confocal microscope at a x20 objective. Single channel Iba1 and nuclear stain are presented along with a merge of both channels for mice treated with ACSF or CCL2. (**B-C**) Microglia cell count from the cortical area (B) and hilus (C) of the dentate gyrus. Bars represent mean ± SEM, two-way ANOVA with Tukey multiple comparison tests. N=3 mice per group, with 4 hippocampal slices measured and averaged per mouse.

4.3 Discussion

Immune factors that play a critical role in the peripheral immune system, such as cytokines and chemokines, are now known to be produced by cells of the peripheral and the central nervous system (CNS) and play important roles in normal CNS functions as well as pathological states (Glabinski and Ransohoff, 1999; Banisadr *et al.*, 2005c; Arisi, 2014). The primary source of neuroimmune factors in the CNS are glial cells, astrocytes, and microglia (Smith *et al.*, 2012; Jensen, Massie and De Keyser, 2013; Choi *et al.*, 2014). Production of neuroimmune factors in the CNS is generally low but increases significantly during injury, disease, and aging, when neuroimmune factors are thought to play important protective and repair roles. However, if the production of neuroimmune factors becomes dysregulated, the elevated levels may promote pathological processes associated with disease, rather than regulate homeostatic functions and amending negative effects of any adverse processes (Gruol, Vo and Bray, 2014). Dysregulation of cytokines and chemokines during aging and disease alters neuronal function and behaviour (Goshen and Yirmiya, 2009), and pro-inflammatory mediator increase in the hippocampus has been shown to impair hippocampal-dependent learning and spatial memory (Gibertini *et al.*, 1995; Barrientos *et al.*, 2002).

CCL2 is a major pro-inflammatory chemokine that increases systemically in normal aging and can cross the blood brain barrier and exert its pro-inflammatory effects in the brain (Deshmane *et al.*, 2009). CCL2 is also produced by brain cells, particularly microglia, either in a constitutive manner, or in response to infection or injury (Deshmane *et al.*, 2009), and is expressed in CNS neurons in the hypothalamus, substantia nigra, cerebellum and hippocampus (Banisadr *et al.*, 2005b; Gosselin *et al.*, 2005). CCL2 directly enhances neuronal excitability and synaptic transmission in the rat hippocampus by binding to its cognate receptor CCR2 (Zhou *et al.*, 2011) and elevated levels are associated with a faster rate of cognitive decline (Westin *et al.*, 2012) and contribute to the pathophysiology of neurodegenerative diseases including Alzheimer's disease and multiple sclerosis (Mahad and Ransohoff, 2003; Ishizuka *et al.*, 1997; Bettcher *et al.*, 2016). In this chapter I investigated whether CCL2 affects hippocampal excitability in early aging.

4.3.1 CCL2 increases dentate gyrus excitability

In this chapter I report CCL2-induced increase in the excitability of dentate gyrus neurons during perforant path activation in both juvenile and adult cohorts. The excitatory effects of CCL2 have previously been observed in dorsal root ganglion neurons (White *et al.*, 2005; Sun *et al.*, 2006), lamina II spinal cord neurons (Gao *et al.*, 2009) and CA1 hippocampal neurons (Zhou *et al.*, 2011; Duan *et al.*, 2018), indicating that CCL2 increases neuronal activity throughout the central nervous system.

The mechanisms underlying the CCL2-induced changes that I have reported here are currently unclear. Measuring the intrinsic properties of granule neurons before and during CCL2 addition may help to uncover these mechanisms. Previous work showed that CCL2-induced increases in the amplitude of excitatory post synaptic currents (EPSCs) in CA1 neurons can be blocked by AMPA and NMDA receptor antagonists (Gao *et al.*, 2009; Duan *et al.*, 2018) suggesting that CCL2 modulates hippocampal excitatory transmission through postsynaptic mechanisms. CCL2's excitatory effects on neurons could also involve presynaptic mechanisms. The frequency of mini EPSCS (mEPSCs) depends on the probability of releasing excitatory neurotransmitters from pre-synaptic terminals and/or number of functional synapses (Del Castillo and Katz, 1954), whereas EPSC amplitude is dependent on the amount of neurotransmitter released, post-synaptic sensitivity and driving force for ions conducting the EPSCs (Van der Kloot, 1991). Previous work has shown that CCL2 depolarises the neuronal membrane of CA1 hippocampal neurons and increases frequency of spontaneous EPSCs without altering EPSC amplitude (Zhou *et al.*, 2011), which the authors suggest could indicate CCL2 enhancement of EPSCs via pre-synaptic transmission. A similar CCL2-induced increase in mEPSC frequency but not mEPSC amplitude in CA1 neurons is reported by Duan *et al*; 2018, however the authors also reported that paired-pulse ratio, a measure of presynaptic release probability, was not affected (Duan *et al.*, 2018). In spinal cord slices the CCL2-induced increase in the frequency of mEPSCs was accompanied by larger amplitudes of NMDA input currents (Gao *et al.*, 2009). Thus, CCL2 could be exerting effects on hippocampal excitatory synaptic transmission through both pre- and post-synaptic mechanisms. Future work aimed at measuring the intrinsic properties of granule neurons before and during CCL2 addition may help to further understand how CCL2 increased granule cell excitability in my experiments.

4.3.1.1 Hippocampal synaptic transmission is dependent on CCL2 concentration and incubation duration

The experiments reported in this thesis used recombinant CCL2 at 7 nM (100ng/ml). Previous electrophysiology studies that have assessed effects of CCL2 on neuronal excitability used acute concentrations ranging from 2.3 nM to 100 nM (Gruol *et al.*, 2014; Guyon *et al.*, 2009; Zhou *et al.*, 2011; Belkouch *et al.*, 2011). It is difficult to determine whether the acute concentrations of CCL2 used in these studies and in my work reflect levels of CCL2 that arise during injury or disease *in vivo*. Studies on traumatic brain injury, where brain inflammation contributes to damage and pathology, showed that injury to the cortex in rats elicited peak CCL2 levels of 20,000 pg/ml, a 4 fold increase compared to basal cortical controls (Jiang *et al.*, 2021). This level of CCL2 was also associated with a reduction in spatial learning and memory (Jiang *et al.*, 2021), indicating that low levels of CCL2 can affect hippocampal-dependent function. Future work could involve measuring whether lower concentrations of CCL2 also affect dentate gyrus excitability in my *in vitro* model, using concentrations which are more reflective of CCL2 levels produced during inflammatory responses *in vivo*. Lower concentrations of CCL2 have previously been shown to elicit physiological responses. For example, 20pg/µl CCL2 intranigral injection caused significant dopamine release from substantia nigra neurons and significantly affected locomotor behaviour (Guyon *et al.*, 2009). In contrast, 10ng/ml CCL2 addition to spinal cord neurons via bath application did not elicit changes in lamina II neuron excitability, whereas 100ng/ml did (Gao *et al.*, 2009), suggesting that CCL2 sensitivity is greater in the brain than spinal cord. Previous experiments also highlight that prolonged exposure to CCL2 is required before noticeable neuronal excitability changes are identified. Action potential firing frequency in substantia nigra neurons were only elicited 8 minutes after CCL2 addition and maximum frequency change was reported 40 minutes after incubation (Guyon *et al.*, 2009). Similarly changes in EPSC amplitude in CA1 neurons began 5 minutes after CCL2 treatment, and peak amplitude change was reached after 15 minutes of incubation (Zhou *et al.*, 2011). This indicates that CCL2 does not mediate instantaneous effects on neurons and could instead be exerting its effects through a variety of secondary messenger systems and cross-talk with other inflammatory mediators.

4.3.2 CCL2 effects hippocampal excitability via CCR2

I show that dentate gyrus excitability induced by CCL2 occurs downstream of CCL2 binding to its specific cognate receptor CCR2, as incubating hippocampal slices with 1μM of a specific CCR2 antagonist eliminated CCR2-induced excitability. This concentration of antagonist blocks 60% migration of CCL2-driven chemotaxis (Mirzadegan *et al.*, 2000) in a monocyte cell line.

CCL2 exerting its proinflammatory effects and affecting neuronal excitability through binding to its cognate receptor CCR2 has been extensively reported (Pinke *et al.*, 2013; Deshmane *et al.*, 2009; Duan *et al.*, 2018; White *et al.*, 2005). It is important to note that other pro-inflammatory chemokines such as CCL7, CCL8, CCL13 and CCL17 can bind to CCR2 and generate down-stream affects (Proudfoot, 2002; Wells *et al.*, 2006), however they bind with a much lower affinity than CCL2 (Zhang, Patel and Pienta, 2010) and none of the above chemokines have been associated with hippocampal excitability. CCL2 application onto CCR2 homozygous knockout mice causes no changes in CA1 neuron EPSC excitability, and RNA interference of CCR2 blocks CCL2-induced increase in pyramidal neuron EPSC frequency (Duan *et al.*, 2018). CCR2 is a G-protein-coupled receptor that, once activated via CCL2 binding, hydrolyses inositol phospholipid 2 (PIP2) into membrane-bound diacylglycerol and soluble inositol triphosphate (IP_3). Generation of IP_3 results in inositol triphosphate receptor-mediated release of Ca^{2+} from intracellular stores and Ca^{2} influx downstream triphosphate signalling (Kuang *et al.*, 1996; Wu, Huang and Jiang, 2000). Calcium influx initiated downstream of CCR2-CCL2 binding is regarded as an important mechanism for enhancing neuronal excitability. This has been confirmed by bath application of a phospholipase C (PLC) inhibitor, and intracellular loading of the calcium chelator BAPTA (which blocks physiological calcium responses (Collatz, Rüdel and Brinkmeier, 1997)) into the postsynaptic cell completely blocks the effect of CCL2 enhanced EPSC frequency in CA1 pyramidal neurons (Duan *et al.*, 2018). In addition to demonstrating the importance of calcium in mediating the effects of CCL2, this result provides evidence that CCL2 could be mediating its effects through postsynaptic mechanisms (Duan *et al.*, 2018), and as previously discussed above, suggests CCL2 regulates neuronal excitability via presynaptic and post-synaptic mechanisms.

4.3.3 CCL2's effect on hippocampal excitability is not age dependent

In my experiments CCL2 increased dentate gyrus excitability in both juvenile and adult mice, with no significant age-related effect observed. These results indicate that the hippocampal excitability increase in early aging (reported in Chapter 3) is unlikely to be mediated by CCL2. It is well documented that CCL2 concentration in the circulatory system is up-regulated in an age dependent manner. ELISA experiments conducted on serum samples show that CCL2 concentration increases progressively through the age groups studied; 3, 5, 12, 18 and 22 month old mice (Yousefzadeh *et al.*, 2018). Longitudinal studies in humans show a similar pattern with CCL2 plasma and serum levels increasing with aging (Antonelli *et al.*, 2006; Bettcher *et al.*, 2019). Notably, and CCL2 appears to be one of the only proinflammatory chemokines upregulated gradually with aging, with no changes in concentration of CCL3, CCL4 and CX_3CL1 concentration observed in human serum (Seidler *et al.*, 2010).

While an age-dependent increase in circulating CCL2 levels is well documented, it is unclear whether circulating CCL2 levels affect the brain or whether CCL2 is produced locally by brain cells in an age-dependent manner. It is noteworthy that in my experiments, which show that dentate gyrus sensitivity to CCL2 is not changed in early aging, the hippocampal slices were prepared from perfused brain where the blood is removed. It is also worth highlighting that increased CCL2 has been reported in the brain in late aging: ELISA experiments conducted on mouse hippocampal tissue revealed that CCL2 concentration does not significantly change between juvenile (1-2 month old), young adult (3-5 months old) and adult (7-9 month old) mice (Gruol, Vo and Bray, 2014), yet hippocampal mRNA was significantly greater in aged rats (20-26 months old) compared to adults (3-5 months old) (Blau *et al.*, 2012). These data suggest that CCL2 is upregulated in correlation with aging in the circulatory system, but not in brain tissue, where an increase is only observed in later aging.

4.3.3.1 CCL2 and age-related blood brain barrier disruption

Blood CCL2 increases progressively whereas in brain tissue CCL2 is only upregulated in late aging or in neurological diseases (Ishizuka *et al.*, 1997; Sokolova *et al.*, 2009), and it is not clear whether this arises through an increase in CNS CCL2 production via glial cells or instead via more CCL2 translocating from the circulatory system into brain tissue across the blood-brain barrier (BBB). CCL2 is transported transcellularly across

the blood brain barrier via the interaction with specific carrier molecules such as caveolin-1 (Ge and Pachter, 2004), however there is no evidence whether CCL2 translocation is increased during aging. CCL2 has been shown to have direct effects on BBB permeability, with *in vitro* models highlighting that the cellular components of the BBB, astrocytes, and brain microvascular endothelial cells, undergo significant changes in cytoskeletal structure and tight junction redistribution in response to CCL2 exposure (Song and Pachter, 2004; Dzenko *et al.*, 2005; Stamatovic *et al.*, 2005). This consequently renders the brain more porous and facilitates migration of blood-borne leukocytes into the brain which continue to conduct inflammatory processes (Semple *et al.*, 2010). CCL2 modulation of BBB permeability appears to be mediated via binding to CCR2 expressed on endothelial cells, as tight junction protein distribution and BBB permeability was not altered after CCL2 treatment in CCR2 knockout mice (Stamatovic *et al.*, 2003). Furthermore, only higher concentrations of CCL2 can disrupt BBB function, as 25ng/ml of CCL2 administered via intracerebroventricular injection in rodents caused BBB leakage, whilst 5ng/ml had no effect (Stamatovic *et al.*, 2005). High concentrations of CCL2 have been associated with the pathogenesis of a variety of inflammatory diseases such as rheumatoid arthritis (Stankovic *et al.*, 2009), atherosclerosis (Harrington, 2000) and multiple sclerosis (Mahad and Ransohoff, 2003) and neurodegenerative diseases such as Alzheimer's Disease (Lee *et al.*, 2018; Westin *et al.*, 2012) HIV-related dementia (Thames *et al.*, 2015) and inflammation in response to injury (Xu *et al.*, 2017). Alzheimer's disease progression is positively correlated with CCL2 concentration in the blood and spinal cord (Lee *et al.*, 2018; Westin *et al.*, 2012), and late-stage Alzheimer's patients exhibit more extensive BBB breakdown than aged matched controls with less extensive pathogenesis (Bowman *et al.*, 2019). Increased CCL2 concentration is also associated with cognitive decline. Hippocampal injection of CCL2 significantly reduced spatial-working memory and object-recognition memory in mice (Long *et al.*, 2020) whereas no change in hippocampal-dependent memory was observed in CCL2 knockout mice (Tian *et al.*, 2017). This is supported by human studies indicating that increased CCL2 expression in the cerebral spinal fluid and plasma of patients with Alzheimer's Disease and HIV-associated dementia show CCL2-related cognitive decline (Lee *et al.*, 2018; Thames *et al.*, 2015; Westin *et al.*, 2012).

Systemic inflammation, which involves the upregulation of many proinflammatory mediators, is well documented to impair memory consolidation and spatial-dependent memory (Monje, Toda and Palmer, 2003; Cunningham and Sanderson, 2008; Jiang *et al.*, 2021). However, systemic inflammation induced by tibial fracture surgery specifically increased CCL2 concentration in the hippocampus (Xu *et al.*, 2017), highlighting that the hippocampus has an acute sensitivity to CCL2. These results indicate that peripheral CCL2 influences hippocampal-dependent function, although the mechanisms underlying this phenomenon are not fully understood. BBB breakdown is also observed in healthy aging, with 21-24 month old mice showing greater BBB permeability compared to 4-6 month old mice (Lee *et al.*, 2012), and the aged brain also shows greater BBB disruption after cortical impact injury compared to young (Onyszchuk *et al.*, 2007). However, no changes in BBB permeability are observed in early aging, with no differences observed between mice that are 3, 7 and 12 months old (Pelegri *et al.*, 2007).

CCL2 is upregulated in the blood in early aging (Yousefzadeh *et al.*, 2018), yet its increased incidence in brain tissue is not apparent until late aging (Gruol, Vo and Bray, 2014; Blau *et al.*, 2012). This suggests that the BBB prevents excessive CCL2 in the blood translocating into the brain, justified by low concentration CCL2 having no effects on BBB permeability (Stamatovic *et al.*, 2005), and my observation of no age-related effects on hippocampal excitability. However, during late aging, or in the case of neurodegenerative disease (Antonelli *et al.*, 2006; Bettcher *et al.*, 2019), CCL2 concentration in the blood and spinal fluid are extensively increased, and at high concentrations CCL2 can cause significant BBB disruption (Bowman *et al.*, 2019), which also occurs during late aging (Lee *et al.*, 2012). This disruption allows for greater “leakage” of blood-borne leukocytes and CCL2 into the brain which continue to conduct inflammatory processes (Semple *et al.*, 2010). The hippocampus is acutely and selectively sensitive to CCL2 (Xu *et al.*, 2017), as I show that another proinflammatory mediator CCL5 has no extensive effects on hippocampal activity. Furthermore, CCL2 presence in the brain at high concentrations specifically affect hippocampal-dependent behaviour and have been shown to be involved in cognitive decline.

4.3.4 CCL2 has limited effects on dentate gyrus microglia proliferation and morphology

As an active sensor and monitor in the brain, microglia are neuroprotective. However, dysregulated microglia response may be dangerous to the survival of injured neurons or even cause damage to healthy neurons that are afflicted by excessive inflammation (Luo, Ding and Chen, 2010). It is well documented that CCL2 signalling induces microglia activation and proliferation (He *et al.*, 2016; Zhang and De Koninck, 2006), which has been reported to occur in the hippocampus (Tian *et al.*, 2017). CCR2 signalling has been found to be essential for CCL2-induced microglia activation, as intrathecal injection of CCL2 elicited no changes in microglia in CCR2 knockout mice (Zhang *et al.*, 2007; Thacker *et al.*, 2009).

In this chapter I report an increase in microglia branch length in hippocampal slices treated with CCL2 compared to controls. Resting microglia express a ramified morphology, composed of long branch processes and a small cell body (Kreutzberg, 1996). During activation, microglia undergo rapid de-ramification, where microglia express larger cell bodies, shorter and thicker processes with extensive branching (Fernandez-Arjona *et al.*, 2017; Davis, Foster and Thomas, 1994). Therefore, increased branch length after CCL2 treatment suggests that microglia are undergoing ramification of microglia morphology to a de-activated state, which contradicts current literature observing CCL2-mediated microglial activation (Zhang *et al.*, 2017).

Despite this, the experiments in this chapter indicated that Iba1-stained microglia in the dentate gyrus showed no changes in cell number after 1 hour incubation with 7 nM CCL2. This period of incubation is relatively short to assess proliferation given that CCL2 has been described to induce IBA1 microglia activation via transcriptional regulation, which takes hours to manifest (Gao *et al.*, 2009). Longer incubation time with CCL2 could reveal different results. Papers reporting CCL2-dependent increased microglial activation/proliferation, treated rodents with a series of CCL2 injections over a period of days (Zhang *et al.*, 2007; Gao *et al.*, 2009; Thacker *et al.*, 2009). However, similarly to my results, a shorter incubation of 24 hour CCL2 treatment did not induce morphological changes in cultured neurons (Hinojosa *et al.*, 2011

), highlighting that microglial activation in response to CCL2 is not instantaneous.

4.3.4.1 Microglia number and morphology does not change in early aging

During injury, microglia cells change their morphology dramatically, migrate to the lesion sites, and proliferate. Proliferated microglia act to phagocytose dying cells and other debris and release cytokines and chemokines to maintain the microenvironment homeostasis and support injured neurons and are beneficial for the neuronal survival. However, mounting evidence has implicated neurotoxic roles of microglia when over-activated in severe injury or neurodegenerative diseases (Luo, Ding and Chen, 2010). Microglia function and morphology also change during aging, becoming senescent. Microglia senescence renders microglia to function abnormally, to fail to respond correctly to stimuli (Sawada, Sawada and Nagatsu, 2008; Conde and Streit, 2006) and, eventually, to promote neurodegeneration. The most prominent, and first identified feature of microglia senescence is the morphological alteration described as "dystrophy". Characteristics of dystrophic microglia observed in the aged brain include de-ramification, cytoplasmic beading and spheroid formation, shortened and twisted cytoplasmic processes, and instances of partial or complete cytoplasmic fragmentation (Streit *et al.*, 2004). Such dystrophic microglia were prevalent and extensively distributed in the brain of older human subjects (Streit *et al.*, 2004; Wasserman, Yang and Schlichter, 2008), whereas normal ramified microglial morphology with only rare instances of dystrophic microglia are seen in the young brain (Conde and Streit, 2006).These observations provide initial evidence of the age-associated changes in microglia in the healthy elderly brain. However, these studies compare young mice with very old (15-30 months of age), and there is sparse literature studying the effects of early aging. The results in this chapter indicate that microglia proliferation and morphology are not affected during early aging, strongly indicating that microglia senescence is a pathological hallmark of late aging.

4.4 Future work

The work in this chapter indicates that CCL2 significantly increased hippocampal excitability, whereas the proinflammatory chemokine CCL5, does not enhance excitability to the same extent. This suggests that the hippocampus is not equally sensitive to all proinflammatory chemokines. The chemokines $CXCL_3$ and CCL3 have been shown to be upregulated in the hippocampus in disease and affect cognition (Parachikova, Nichol and Cotman, 2008; Minogue *et al.*, 2014; Nicaise *et al.*, 2020).

Future work could test these chemokines on hippocampal function and explore any intrinsic mechanisms relating to any changes in excitability to determine if the hippocampus is specifically sensitive to CCL2, or whether a number of pro-inflammatory chemokines work cumulatively to affect hippocampal activity.

I show that CCL2 increases dentate gyrus excitability, however this was examined using a concentration of CCL2 which is substantially higher than natural levels observed in *in vivo* models of inflammation. As hippocampal sensitivity is shown to be positively correlated to CCL2 concentration, future work could examine whether lower concentrations of CCL2 also elicit significant increases in hippocampal excitability. Furthermore, investigating the intrinsic properties of cells in the dentate gyrus granule cell layer may help to expose mechanisms responsible for CCL2-dependent dentate gyrus excitability. Several intrinsic experiments conducted on a variety of neuronal subtypes including CA1 pyramidal cells, have indicated that CCL2 enhances neuronal activity via excitatory synaptic transmission, although it is not clear whether this occurs through pre or post-synaptic activity, or a combination of both. Intrinsic properties such as EPSP generation to perforant path activation, resting membrane potential and membrane resistance in response to CCL2 addition could be examined using *in vitro* intracellular recording techniques as described 3.2.2. As controversy exists as to whether CCL2 mediates via pre- or post-synaptic mechanisms in CA1 neurons, it could be valuable to examine these properties in dentate gyrus neurons. The frequency of EPSPs depends on the probability of releasing excitatory neurotransmitters from pre-synaptic terminals and number of functional synapses (Del Castillo and Katz, 1954), whereas EPSC amplitude is dependent on the amount of neurotransmitter released, post-synaptic sensitivity and driving force for ions conducting the EPSCs (Van der Kloot, 1991). Therefore, measuring fEPSP frequency and amplitude upon perforant path activation through repetitive burst stimulation could help to examine synaptic transmission properties.

Reports in the literature highlight that whilst CCL2 concentration increases in correlation with age in the blood, no differences are observed in early aging in hippocampal tissue. However, these experiments were not performed on matched age cohorts or from blood and brain tissue from the same animal. Future work could involve conducting ELISAs on blood, cerebral spinal fluid and hippocampal tissue from juvenile (9-15 week old) and

adult (25-35 week old) mice, to examine how CCL2 concentration changes during early aging.

A plethora of evidence describes that CCL2 causes microglia activation and proliferation in the brain, and microglia morphology is reported to change in normal aging. The results described in this chapter indicate little change to microglia activation in response to age or CCL2, however this could be a result of the low incubation time of CCL2. Significant microglia responses have all been elicited in the rodent brain after CCL2 administration over a period of days, and alternatively, a shorter incubation of 24 hours reported in the literature also elicited no changes in microglia activation (Stamatovic *et al.*, 2005). Additionally, I incubated hippocampal slices in CCL2 in *in vitro* conditions, with no circulatory system present. This administration of CCL2 did not represent conditions of brain *in vivo* accurately, which could also have accounted for lack of microglia activation identified. In future it would be better to administer CCL2 in in vivo rodent models, potentially via intrathecal injection as described in (Long *et al.*, 2020), over a period of a few days to ensure sustained treatment. CCL2 visualisation through immunohistochemical Iba1 staining did generate obvious microglial immunofluorescence for analysis, however due to high background staining there was a lack of clarity in some sections. To improve microglial analysis, future work could involve using green fluorescent protein (GFP) labelled microglia using CX3CR1-GFP transgenic mice (Wolf *et al.*, 2013), which generally generates clean and strong microglial staining.

CCL2 has been described to directly affect hippocampal-dependent memory, yet there has been little investigation into whether CCL2 affects cognition in an age-dependent manner. As CCL2 increases in the blood with healthy aging, it would be valuable to examine whether this increase in concentration affects cognition throughout life. This could help to identify whether dysregulation of CCL2 is a specifically responsible for age-related cognitive decline. These experiments could be conducted by treating cohorts of mice with different concentrations of CCL2, administering via bilateral hippocampal injection and examining their behavioural scores in hippocampal-dependent cognitive tests, such as the Morris water maze, Y-maze spontaneous alternation task or object recognition studies. Comparing these outcomes to control groups could help to identify changes in hippocampal-dependent function. Additionally, performing these experiments

on microglial-GFP labelled mice would help to investigate whether a direct link between microglia activation, CCL2 and cognition exists.

4.5 Summary

The work in this chapter showed that 7 nM CCL2 significantly increased excitability in the dentate gyrus via its cognate receptor CCR2. The proinflammatory chemokine CCL5 did not enhance hippocampal excitability to the same extent as CCL2, which suggests that the hippocampus is not equally sensitive to all proinflammatory chemokines. Future work aimed at measuring the intrinsic properties of granule neurons before and during CCL2 addition may help to further understand how CCL2 increased granule cell excitability in my experiments.

CCL2 increased dentate gyrus excitability in both juvenile (9-15 weeks old) and adult (25-35 weeks old) mice, with no significant age-related effect observed. These results indicate that the hippocampal excitability increase in early aging, as reported in Chapter 3, is unlikely to be mediated by CCL2.

In this chapter I report an increase in microglia branch length in Iba1-stained hippocampal slices treated with CCL2 compared to controls. Increased branch length after CCL2 treatment suggests that microglia are undergoing ramification of microglia morphology to a de-activated state. No changes in microglia number after CCL2 incubation were reported, however. Additionally, microglia proliferation and morphology were not affected in the dentate gyrus during early aging, strongly indicating that microglia senescence is a pathological hallmark of late aging.

Chapter 5 – The Duffy antigen receptor for chemokines (DARC) modulates hippocampal function

5.1 Introduction

The Duffy antigen receptor for chemokines (DARC) is a glycosylated membrane protein expressed on erythrocytes and endothelial cells of the spleen, lung, thyroid, kidney, and brain (Hadley and Peiper, 1997; Lee *et al.*, 2003). DARC binds to a number of pro-inflammatory chemokines of the C-C and C-X-C family (Gardner *et al.*, 2004). The binding of chemokines to DARC does not lead to G-protein recruitment due to the absence of an Asp-Arg-Tyr consensus motif in its second cytoplasmic loop. Thus, DARC does not signal when bound to chemokines and does not directly act to translocate effector cells to sites of inflammation like traditional chemokine receptors (Pruenster *et al.*, 2009; Hadley and Peiper, 1997). Nevertheless, DARC has important cell type-dependent biological functions.

DARC expressed on erythrocytes participates in physiological scavenging of chemokines and can mediate chemokine concentration in the blood. Unlike other atypical chemokine receptors, chemokines are not degraded after their internalisation by DARC, instead the receptor acts to remove surplus plasma chemokines that could mediate extensive and harmful inflammation (Mangalmurti *et al.*, 2009; Ransohoff, 2009). Administration of systemic lipopolysaccharide (LPS) to DARC knockout mice leads to a marked increase in neutrophil infiltrate in the lungs and livers compared to wild-type controls (Dawson *et al.*, 2000). These results suggest that DARC plays an important role in chemokine homeostasis.

DARC expressed on endothelial cells internalises and transcytoses chemokines from their sites of synthesis to the luminal side of the endothelium. Here, the chemokines are embolised and contribute to leukocyte extravasation to tissues (Pruenster *et al.*, 2009). Neutrophil and monocyte migration towards cognate chemokines is enhanced in monolayers expressing DARC (Lee *et al.*, 2003) and chemokine injections into mice over-expressing endothelial DARC have significantly greater leukocyte recruitment (Pruenster *et al.*, 2009). These experiments highlight that DARC on endothelial cells acts to mediate cellular transport of chemokines, and also prevents the escape of soluble tissue-derived chemokines into circulation (Zarbock *et al.*, 2010). Inflammation can further up-regulate DARC expression in veins and post-capillary venules and induce DARC to appear in vascular segments that are usually devoid of the receptor (Miller *et al.*, 1975; Segerer *et al.*, 2000; Patterson *et al.*, 2002; Geleff *et al.*, 2010).

The proinflammatory chemokine CCL2 readily binds to DARC, although the biological purpose of this function is not clear. DARC appears to affect circulating CCL2 concentration as knockout mice have reported one-third lower basal CCL2 plasma levels compared to wild-type controls (Fukuma *et al.*, 2003). Humans lacking DARC expression on erythrocytes, have significantly lower basal CCL2 levels than DARC-positive humans (Jilma-Stohlawetz *et al.*, 2001).

DARC also seems to play an important role in brain function. For example, DARC shuttles CCL2 and CCL5 across the blood-brain barrier (BBB) from the basolateral to the luminal side of mouse brain endothelial cells (Minten *et al.*, 2014). Indeed, loss of DARC impairs the transport of CCL5 and CCL2 following TNFα-mediated stimulation of endothelial cells in the brain (Minten *et al.*, 2014). These data suggest that DARC has a pro-inflammatory function in endothelial cells of the brain by shuttling chemokines across the blood-brain barrier. DARC's immunoreactivity has also been observed at high levels in cerebellar Purkinje cells (Horuk *et al.*, 1996), and throughout the cortex in post-mortem brain tissue (Minten *et al.*, 2014).

DARCs role in regulating neuronal function is unknown. Therefore, I aim to investigate whether DARC plays a role in mediating hippocampal function. As DARC controls circulating CCL2 concentration through DARC-CCL2 receptor binding, I also aim to examine whether DARC is responsible, in some part, for mediating CCL2's effect on hippocampal excitability. As DARC has a chemokine scavenging function and regulates chemokine concentration in the blood (Mangalmurti *et al.*, 2009; Ransohoff, 2009), I hypothesised that DARC could be mediating CCL2's effect on hippocampal excitability.

5.2 Results

5.2.1 DARC mediates age-related hippocampal excitability

There have been few experiments studying DARC in the brain, however its expression has been reported on Purkinje cells in the cerebellum and throughout the cortex (Horuk *et al.*, 1997; Minten *et al.*, 2014). I first investigated DARC expression localisation in non-myeloid and myeloid brain cells using single cell RNA sequencing data from the Tabula Muris database (Schaum *et al.*, 2018). This data highlighted that DARC is expressed almost exclusively in neurons (Fig 5.1), suggesting that DARC could play a role in neuronal function.

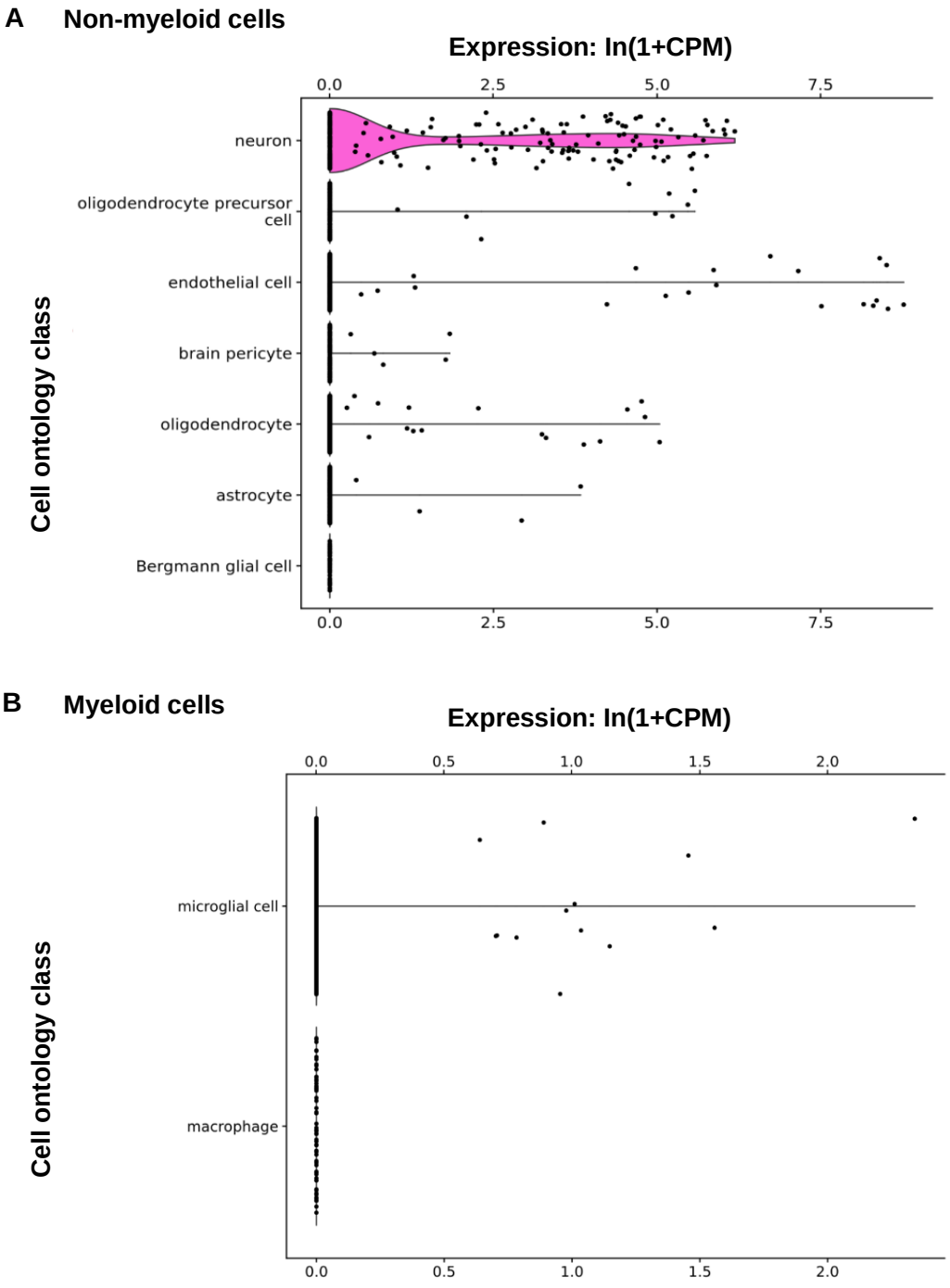


Figure 5.1 DARC is almost exclusively expressed on neurons in the brain. Data mining of single cell RNA sequencing of DARC expression in non-myeloid (A) and myeloid (B) brain cells was conducted using the Tabula Muris single-cell transcriptome database (Schaum *et al.*, 2018). RNA-sequencing was performed on single cells dissociated and isolated by FACs from the cortex, hippocampus, cerebellum and striatum of 10-15 week old mice. Data is shown as gene counts log-normalised for each cell using the natural logarithm of 1+ counts per million (CPM).

Investigation into DARCs occurrence in the brain consists mainly of RNA sequencing experiments or immunohistochemical analysis detecting regional expression. There has

been no investigation into whether DARC is biologically functional, despite its confirmed prevalence on cell bodies in specific neuronal subtypes (Horuk *et al.*, 1997). Proinflammatory chemokines have a direct effect on hippocampal function (as shown in figure 4.1) (Meucci *et al.*, 1998), and DARC acts to regulate chemokine concentration and their function (Mangalmurti *et al.*, 2009; Ransohoff, 2009). This raises the question whether DARC is also able to mediate chemokine-related function in neurons.

I have previously shown that early aging affects hippocampal activity (section 3.2.1), and proinflammatory chemokines such as CCL2 are able to influence this excitability (section 4.2.1). However, the underlying mechanisms responsible for these excitability changes remain elusive. Given that DARC, an atypical receptor for CCL2, is expressed on neurons, I investigated whether DARC could play a role mediating hippocampal function along the perforant pathway. A DARC homozygous knockout mouse model was used, and hippocampal excitability was assessed by conducting the experiments similar to those done in previous chapters.

I first compared dentate gyrus excitability in juvenile and adult DARC knockout mice. Hippocampal slices were prepared from juvenile (9-15 week old) and adult (25-35 week old) DARC knockout mice. Once slices had acclimatised to the *in vitro* conditions, the perforant pathway was activated by electrically stimulating the entorhinal cortex layer II from 5V to 100V and subsequently generated field EPSPs (fEPSPs) were recorded from the dentate gyrus. The rising slope of fEPSPs were measured and plotted against entorhinal cortex stimulation input. The results indicate that dentate gyrus excitability in DARC knockout mice was not affected by early aging in either the supra-pyramidal or infra-pyramidal subregions (Fig 5.2 C). This is a stark contrast to the age-dependant increase in dentate gyrus excitability observed in wild-type mice, especially in the supra-pyramidal subregion, which showed large changes in excitability with early aging (Fig 3.1 C). By directly comparing wild-type and DARC knockout fEPSP values, it is evident that supra-pyramidal dentate gyrus excitability is significantly smaller in DARC knockout adult mice compared with wild-type adult mice (80V: DARCko 0.621 ± 0.029V vs wild-type 2.404 ± 0.755V, P=0.079. 90V: DARCko 0.710 ± 0.303V vs wild-type 2.788 ± 0.751V, P=0.0079. 100V: DARCko 0.777 ± 0.318V vs wild-type 2.822 ± 0.777V, P=0.0096, Fig 5.3). These results suggest that DARC is, in part, responsible for mediating age-related hippocampal excitability.

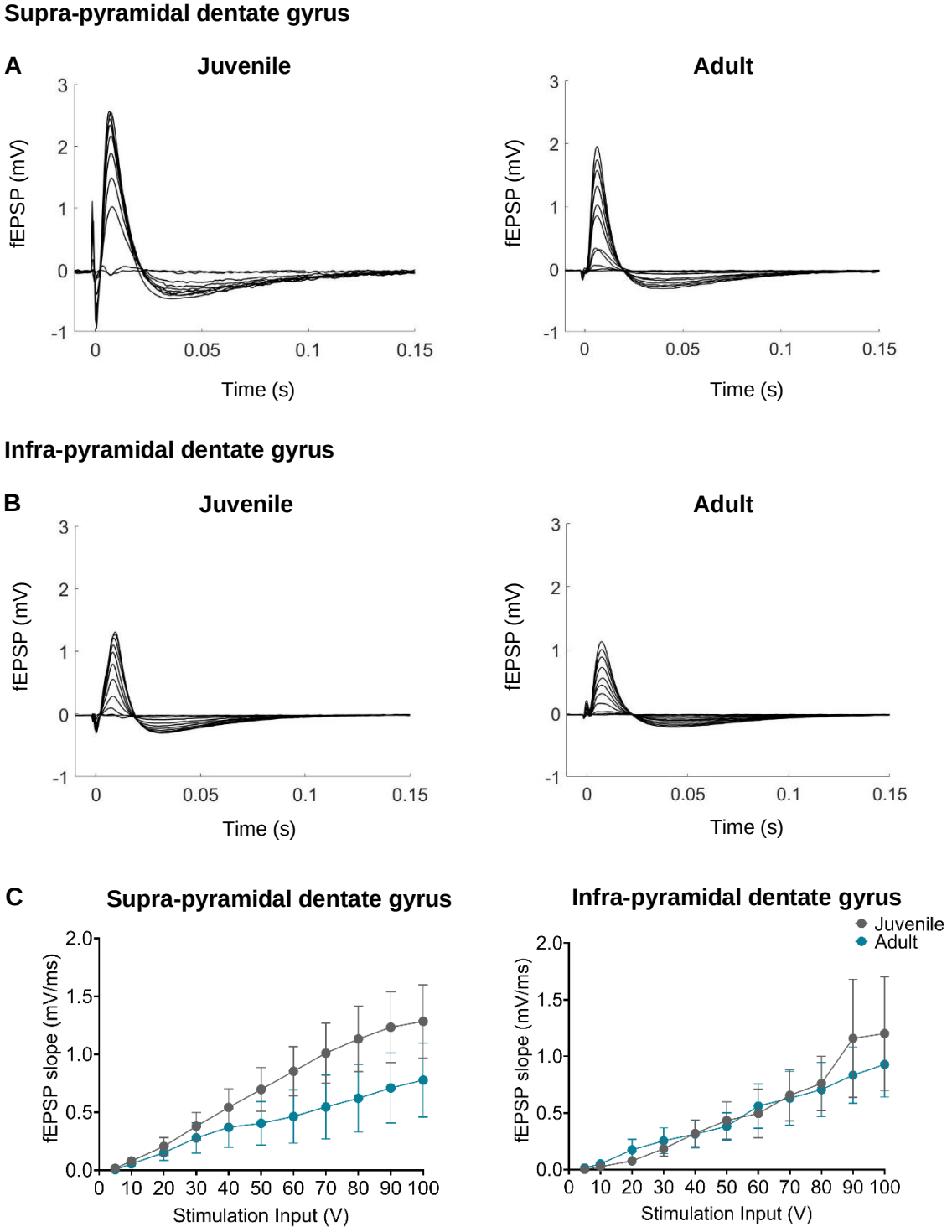


Figure 5.2 Aging has no effect on dentate gyrus excitability in DARC knockout mice. Hippocampal slices were prepared from either juvenile (9-15 weeks old) or adult (25-35 weeks old) DARC knockout mice. The perforant pathway was activated by electrical stimulation of the entorhinal cortex layer II. **(A–B)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal (A) and infra-pyramidal blade (B) of the dentate gyrus. The entorhinal cortex was stimulated at time 0. fEPSPs represent the average response of 10 electrical stimulations (1 Hz) for each intensity (5V–100V). **(C)** Stimulus response curves showing the slope of fEPSPs as a function of stimulation intensity in juvenile and adult mice. Bars represent mean ± SEM. Two-way ANOVA with Sidak multiple comparison tests. N=7 mice for each study group (where N number represents 1 hippocampal slice recording per animal).

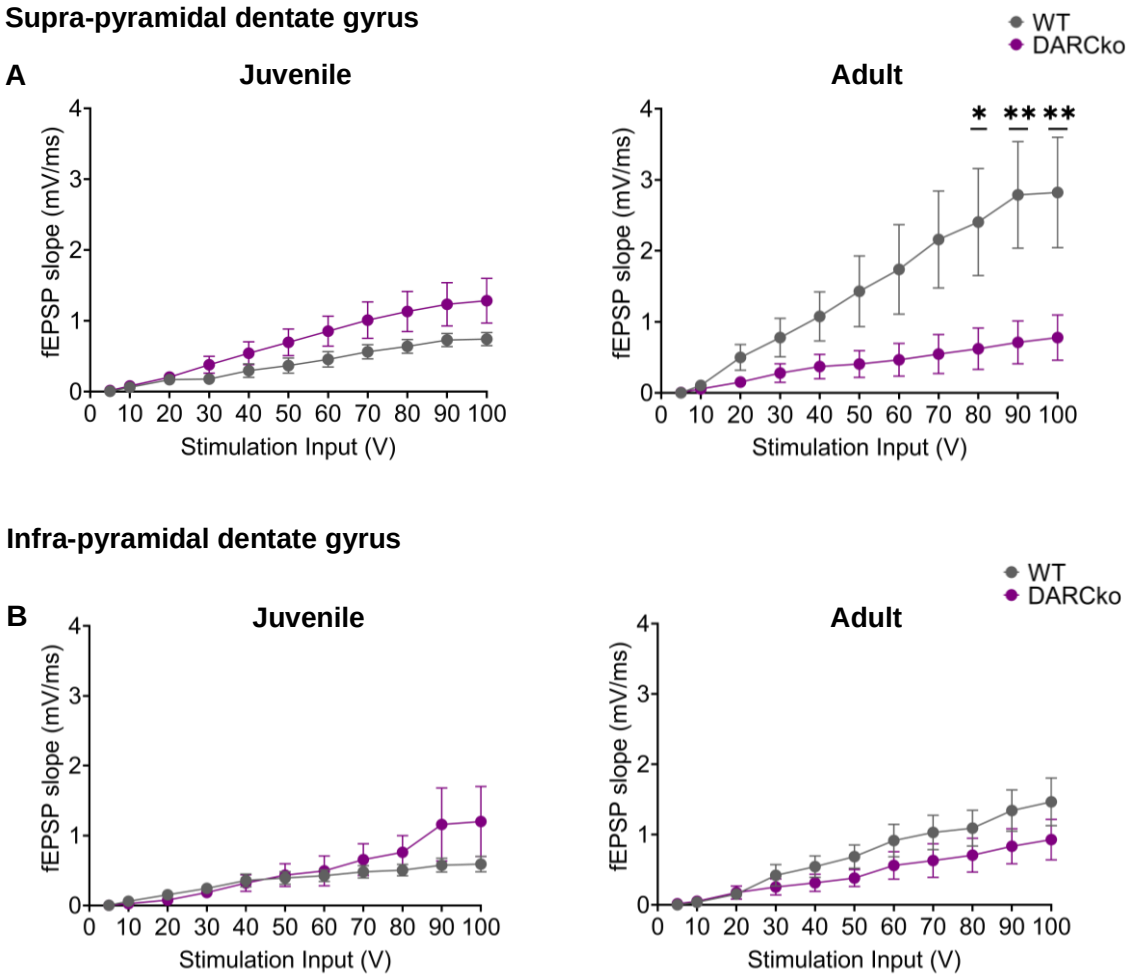


Figure 5.3 Loss of DARC eliminates region-specific age-dependent increase in dentate gyrus excitability. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) wild-type and DARC knock-out mice. The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex. **(A–B)** Stimulus response curves showing the slope of field EPSPs (fEPSPs) as a function of stimulation intensity in wild-type or DARC knock-out mice measured from the supra-pyramidal (A) or infra-pyramidal (B) dentate gyrus. Bars represent mean ± SEM (two-way ANOVA with Sidak multiple comparison tests). * $P \leq 0.05$, ** $P \leq 0.01$, N=6 mice for (1 hippocampal slice per animal) wild-type group and N=7 for DARC knockout study group (where N number represents 1 hippocampal slice recording per animal).

5.2.2 DARC knockout affects granule neuron EPSP strength, resting membrane potential and membrane resistance

This far I have observed through field excitability measurements that age-dependent increase in hippocampal excitability is eliminated in mice lacking the DARC receptor. Notably, dentate gyrus excitability is much lower in adult DARC knockout mice compared to that in wild-type mice. To understand the mechanisms underlying these differences, I investigated intracellular properties of dentate gyrus granule neurons in adult DARC knockout mice using the same methods as described in 3.2.2.

I first examined whether the absence of DARC affected granule neurone EPSP strength upon perforant pathway stimulation. The perforant pathway was activated by electrical stimulation of the entorhinal cortex layer II at 50% maximum and 100% maximum stimulation, and the amplitude of subsequent EPSPs were measured from individual supra-pyramidal and infra-pyramidal dentate gyrus granule neurons in adult mice.

EPSP amplitudes generated by 50% maximum entorhinal cortex stimulation were smaller in DARC knockout mice than wild-type mice in a subregion specific manner. EPSPs measured from supra-pyramidal granule neurons were approximately two fold greater in wild-type mice compared to DARC knockout mice (wild-type mice 20.43 ± 2.317µV vs DARC knockout mice 9.868 ± 2.078µV, P=0.0043) however no difference was observed in the infra-pyramidal subregion (wild-type mice 13.32 ± 2.582µV vs DARC knockout 12.77 ± 2.856µV ns), Fig 5.4 C. A caveat of this experiment is that EPSP amplitude is dependent on the resting membrane potential of the cell. Any significant changes in resting membrane potential, will affect EPSP closeness to the reversal potential and therefore the amplitude of the EPSP recorded.

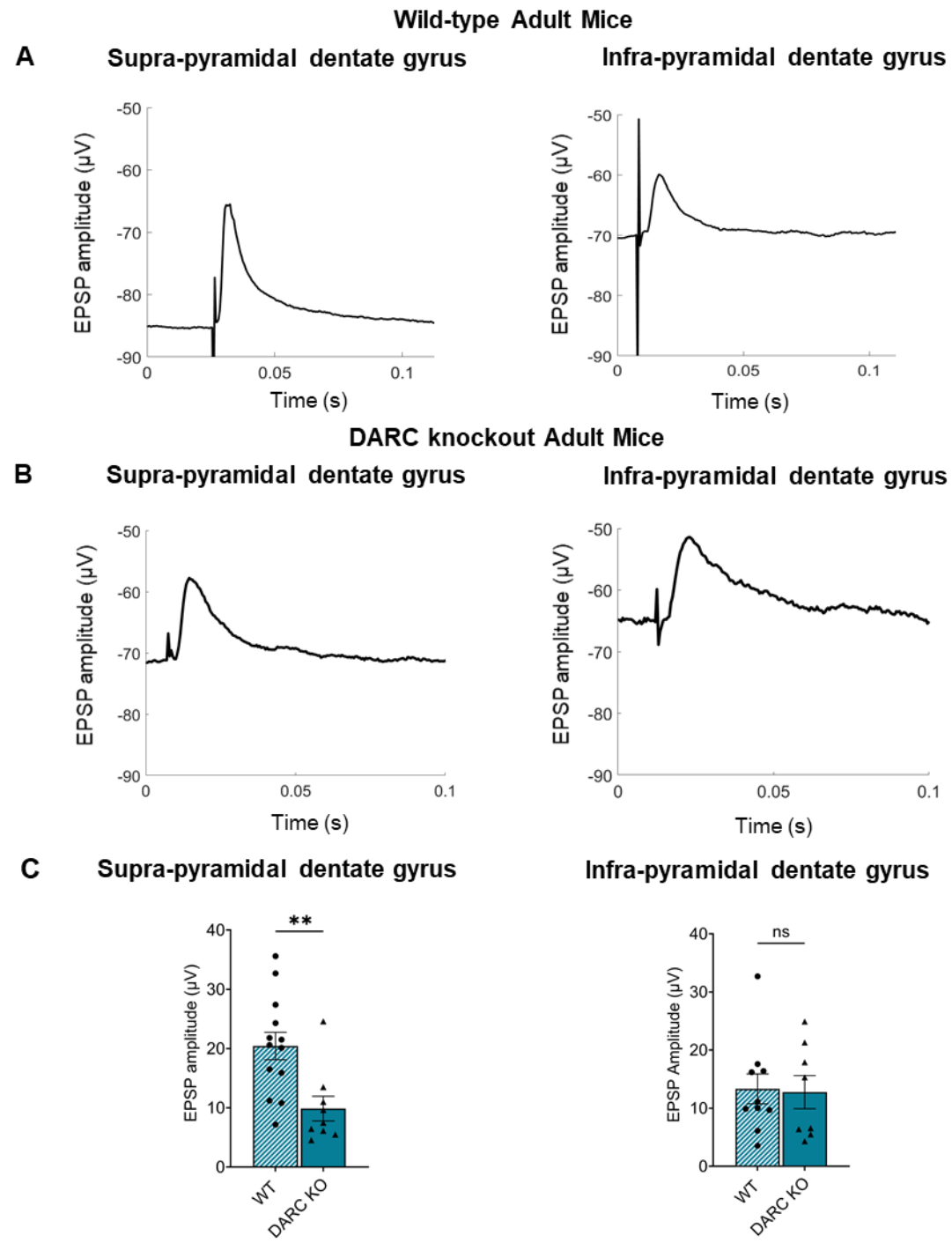


Figure 5.4 Dentate gyrus granule neurones in DARC knockout mice exhibit smaller EPSP amplitudes than wild-type mice in a region specific manner. Hippocampal slices were prepared from either wild-type or DARC knockout adult mice (25-35 weeks of age). The perforant path was activated by electrical stimulation of the entorhinal cortex layer II at 50% maximum stimulation. (**A–B**) Representative EPSPs from the supra-pyramidal and infra-pyramidal blade of the dentate gyrus in wild-type (A) and DARC knockout adult mice (B) stimulated at 50% maximum voltage. (**C**) Grouped data of the average EPSP amplitude recorded from supra-pyramidal and infra-pyramidal granule cells in adult wild-type and DARC knockout adult mice. Bars represent mean ± SEM (Mann-Whitney test), ** $P \leq 0.01$. N=13 and N=9 cells for supra-pyramidal wild-type and DARC knockout mice respectively, and N=10 and N=8 cells for infra-pyramidal wild-type and DARC knockout mice respectively.

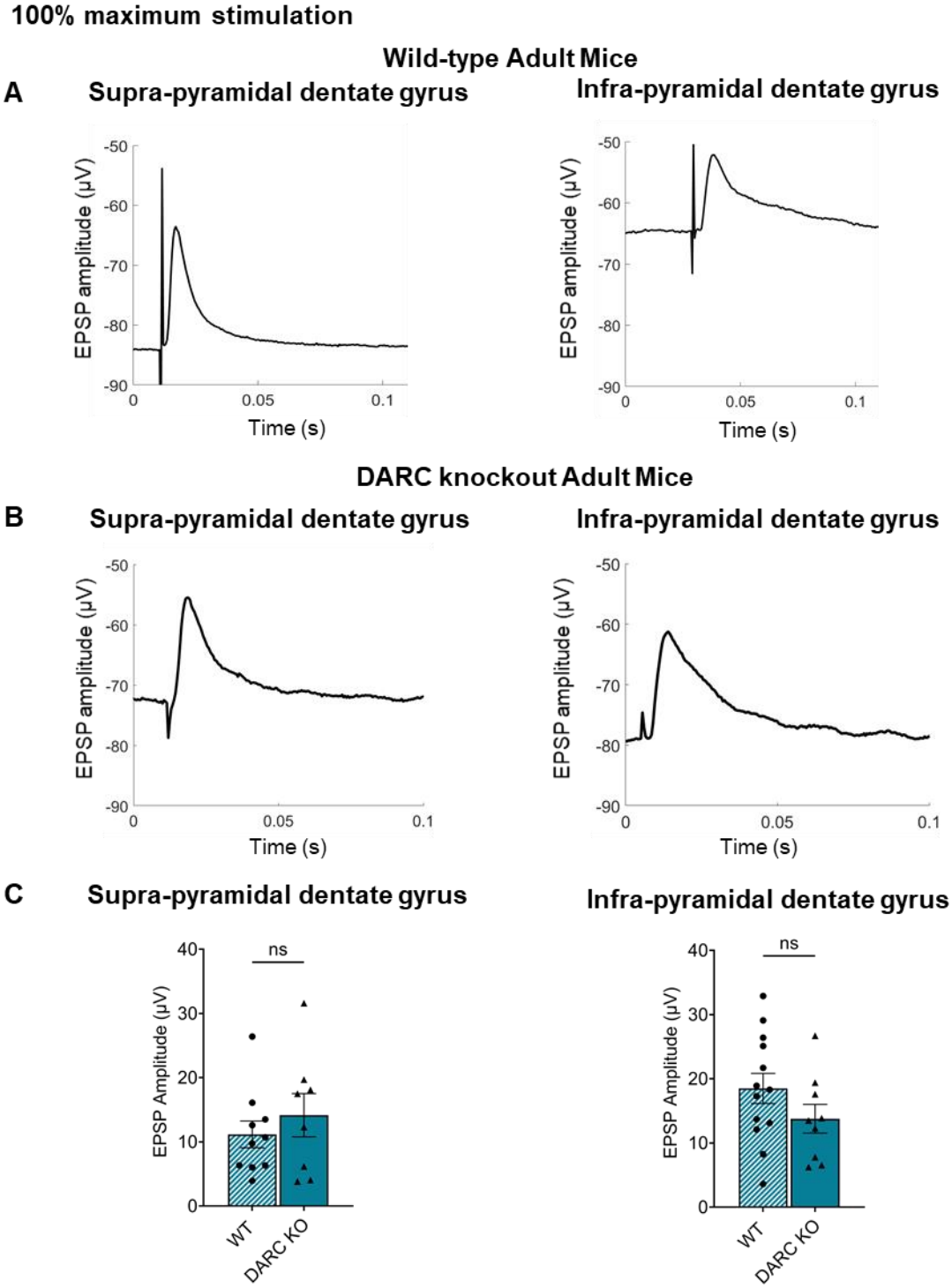


Figure 5.5 The absence of DARC has no effect on dentate gyrus granule neurone EPSPs when the entorhinal cortex is stimulated at maximum. Hippocampal slices were prepared from wild-type and DARC knockout adult mice (25-35 weeks of age). The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex at 100% maximum stimulation. (**A–B**) Representative EPSPs from the supra-pyramidal and infra-pyramidal blade of the dentate from wild-type (A) and DARC knockout mice (B) stimulated at 100% maximum voltage. (**C**) Mean average EPSP amplitude stimulated at 100% maximum stimulation recorded from supra-pyramidal and infra-pyramidal granule cells in wild-type and DARC knockout mice. Bars represent mean ± SEM (Mann-Whitney test). N=10 and N=8 cells for supra-pyramidal wild-type and DARC knockout mice respectively and N=13 and N=9 cells for infra-pyramidal wild-type and DARC knockout mice respectively.

Factors such as neuron membrane resistance and resting membrane potential can lead to different levels of threshold excitation (Lopez-Rojas and Kreutz, 2016), which could provide an explanation for the differences in EPSP amplitudes measured between wild-type and DARC knockout mice (Fig 5.4). Therefore, I next compared the membrane resistance of supra-pyramidal and infra-pyramidal granule neurons in adult wild-type and DARC knockout mice. This was achieved by piercing a granule neuron and injecting input current step-wise from -0.5 nA to +0.5 nA and measuring the subsequent voltage change in membrane potential. Current–voltage curves were plotted, and Ohm's law was used to calculate membrane resistance.

Figure 5.6 shows that there is no difference in supra-pyramidal dentate gyrus granule neuron membrane resistance between wild-type and DARC knockout mice (wild-type mice, 32.90 ± 1.191µV vs DARC knockout mice, 31.37 ± 1.924µV, ns). However, in the infra-pyramidal subregion, DARC knockout granule neurons had a significantly higher membrane resistance compared to wild-types (wild-type mice, 27.44 ± 1.685µV vs DARC knockout mice, 42.59 ± 2.322µV, P<0.0001).

I next measured whether absence of the DARC receptor is affecting the resting membrane potential of granule neurons. This was conducted by piercing either supra-pyramidal or infra-pyramidal granule neurons of the dentate gyrus with a sharp tip glass electrode and measuring the resting voltage for 1 minute. I identified that supra-pyramidal neurons in DARC knockout mice had a hyperpolarised resting membrane potential than neurons in wild-type mice (wild-type mice, -85.26 ± 1.352µV vs DARC knockout mice, -63.90 ± 1.859µV, P<0.0001). No differences in resting membrane potential were observed in the infra-pyramidal subregion, however (wild-type mice, -68.05 ± 1.598µV vs DARC knockout mice, -72.66 ± 1.876µV, ns), Fig 5.7.

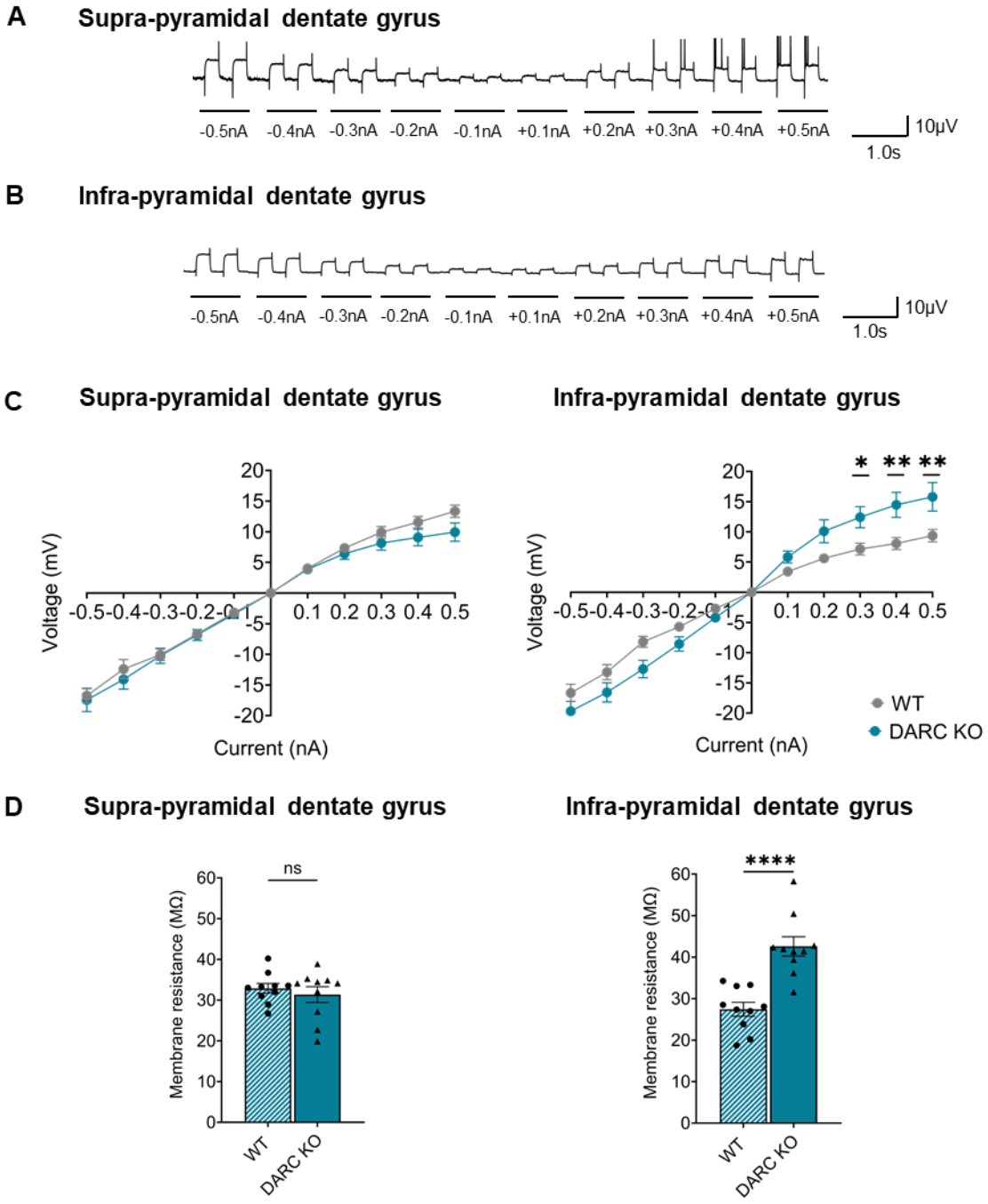


Figure 5.6 Membrane resistance of infra-pyramidal dentate gyrus granule cells is higher in the absence of DARC. Hippocampal slices were prepared from adult (25-35 weeks of age) wild-type mice and DARC knockout mice. Intracellular properties were recorded from supra-pyramidal and infra-pyramidal granule cells of the dentate gyrus. **(A–B)** Representative current-voltage recordings from supra-pyramidal (A) or infra-pyramidal (B) granule cells from wild-type or DARC knockout mice. An input current was injected into cells step wise from -0.5 - +0.5 nA. **(C)** Current-voltage curves showing the voltage output (mV) in response to stepwise input currents (nA). (**D**) Membrane resistance of supra-pyramidal or infra-pyramidal granule neurons in wild-type and DARC knockout adult mice. Bars represent mean ± SEM * P ≤ 0.05, ** P ≤ 0.01, **** P< 0.0001, (C) two-way ANOVA with Sidak multiple comparison tests, (D) Mann-Whitney test. N=10 cells for each group.

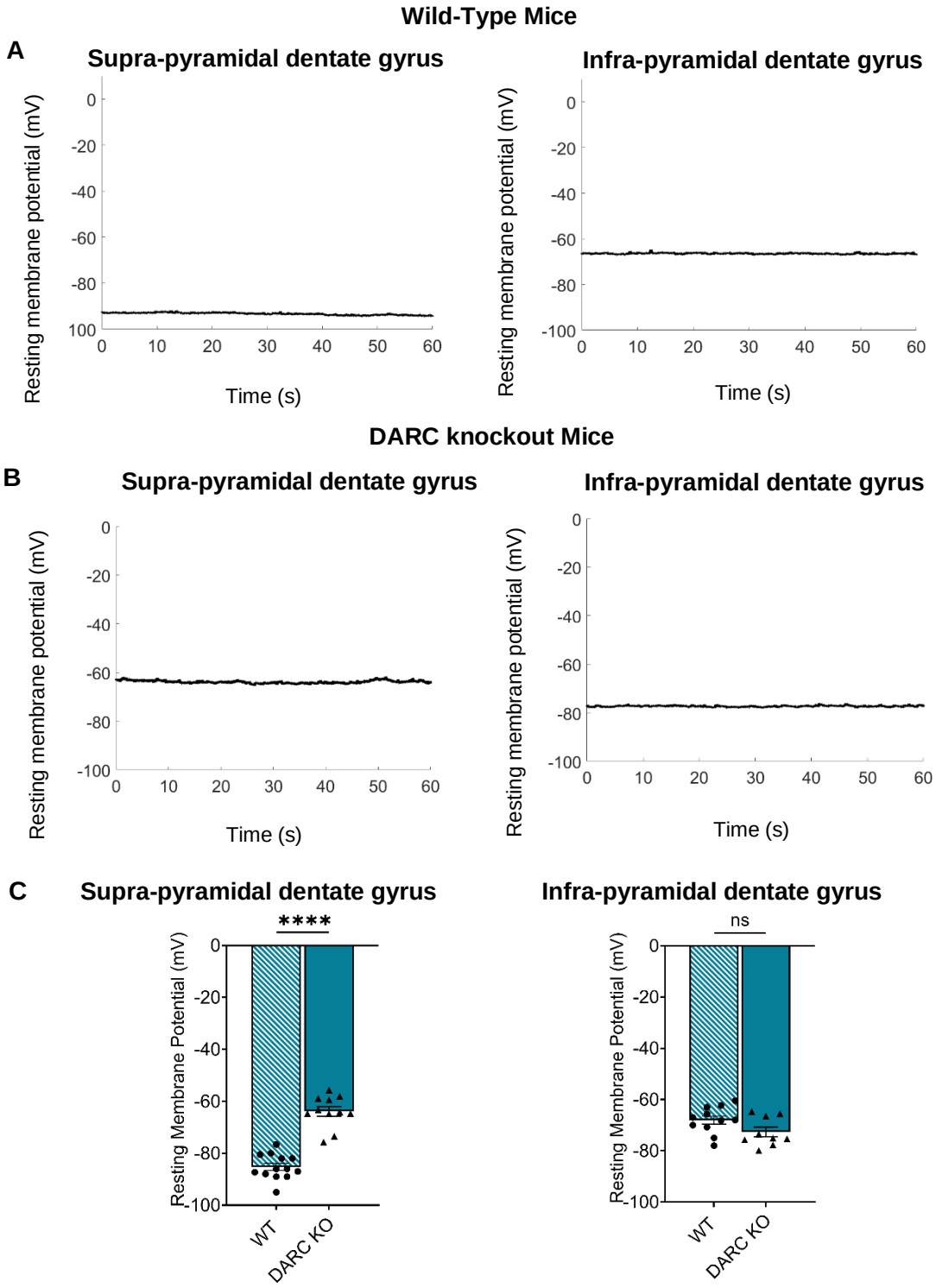


Figure 5.7 The absence DARC affects the resting membrane potential of dentate gyrus granule neurones in a region-specific manner. Hippocampal slices were prepared from wild-type and DARC knockout adult mice (25-35 weeks of age) and resting membrane potential was recorded from supra-pyramidal and infra-pyramidal granule cells of the dentate gyrus. **(A–B)** Representative resting membrane potential traces from supra-pyramidal or infra-pyramidal dentate gyrus granule cells from wild-type (A) or DARC knockout (B) mice. (**C**) Grouped data showing average resting membrane potential of supra-pyramidal (left graph) and infra-pyramidal (right graph) granule neurones in DARC knockout mice compared to wild-type. Data is shown as ± SEM P < 0.0001, Mann-Whitney test. N=13 and N=12 supra-pyramidal cells for wild-type and DARC knockout mice respectively, and N=10 and N=9 infra-pyramidal cells for wild-type and DARC knockout mice respectively.

These results indicate that adult mice lacking the DARC receptor express subtle differences in dentate gyrus cellular properties compared to wild-type aged-matched mice. Specifically, post synaptic responses to perforant pathway stimulation, resting membrane potential and membrane resistance were all affected. This indicates that the DARC atypical receptor is involved in other functions aside from chemokine regulation, potentially acting to regulate neuronal properties in the hippocampus.

5.2.3 Hippocampal gamma oscillations in DARC knockout mice

My data thus far indicates that DARC mediates dentate gyrus excitability, as age-dependent excitability changes in the subregion are diminished in the absence of DARC (Fig 5.2 – 5.3). Further exploration highlights that DARC influences intrinsic membrane properties and postsynaptic responses of granule cell neurons, which could be accounting for the changes in extracellular excitability observed (Fig 5.2 – 5.7). The dentate gyrus is a crucial part of the cortical-hippocampal network that is required for information processing. The entorhinal cortex supplies the major neocortical input to the hippocampus via the perforant pathway (Witter *et al.*, 1989), and the dentate gyrus routes sensory information from the entorhinal cortex to the cornu ammonis (Steward, 1976). For these reasons, I previously investigated if gamma oscillations in the cortical-hippocampal network are affected by early aging. These results demonstrated that although dentate gyrus granule cell properties change in early aging, the gamma oscillatory network remains unaffected (Fig 3.8 – 3.9). Due to the changes in dentate gyrus granule neuron cell properties in DARC knockout mice, I next investigated whether DARC plays a role in cortical-hippocampal gamma activity.

Cortical-hippocampal gamma activity was assessed as previously described in 3.2.3. Hippocampal slices were prepared from juvenile (9-15 weeks old) and adult (25-35 weeks old) DARC knockout mice. Control recordings were taken from the entorhinal cortex and CA3 (Fig 5.8 A and B) before 200 nM of kainic acid was added to the slices via bath application. Induced gamma rhythms were measured from both subregions (Fig 5.8 C and D) and compared to gamma rhythms measured from wild-type mice. I first investigated if DARC is required to maintain gamma oscillation power and frequency in the entorhinal cortex and CA3, and power spectrums were generated to measure these oscillation parameters (Fig 5.8 E and F). Quantification of gamma oscillation power and

frequency in the entorhinal cortex and CA3 highlighted that gamma rhythms are unaffected in DARC knockout mice in both juvenile and adult cohorts, Fig 5.9 A.

I next investigated if DARC plays a role in maintaining synchrony between the entorhinal cortex and CA3. This was conducted by calculating a phase locking value which represents the amount of time the phase of the oscillation is the same between the entorhinal cortex and CA3. In wild-type animals, I have shown there is an increase in the phase relationship between the two subregions at the gamma frequency (~25 Hz) in both juvenile and adult mice (Fig 3.8 E). In DARC knockout mice, there is no significant difference in the phase locking value compared to wild-type mice (the frequency at the phase locking value is: wild-type juvenile mice, 33.1 ± 1.33 Hz; wild-type adult mice, 32.5 ± 1.97 HZ; DARC knockout juvenile mice, 32.9 ± 1.89 Hz; DARC knockout adult mice, 28.4 ± 3.01 Hz, Fig, 5.9 D). When plotting the phase locking values against oscillation frequency, a significant difference in phase locking value is observed between juvenile wild-type and DARC knockout mice between 21.73-27.28 Hz frequencies (21.8 Hz, P=0.0309; 22.73 Hz, P=0.0078; 23.64 Hz, P=0.0027; 24.55 Hz, P=0.0017; 25.46 Hz, P=0.0023; 26.37 Hz, P=0.0049; 27.28 Hz, P=0.0181) and between adult wild-type and DARC knockout mice between 22.73-24.55 Hz frequencies (22.73 Hz, P=0.0418; 23.64 Hz, P=0.025; 24.55 Hz, P=0.043), as shown in Figure 5.9 C.

These data highlight that although DARC is not responsible for mediating amplitude or frequency in the entorhinal cortex and CA3, phase locking appears to be disrupted in the absence of DARC. This implies that synchrony between the entorhinal cortex and CA3, which has been shown to be important in object learning and memory (Fernández-Ruiz *et al.*, 2021), is somewhat mediated by the DARC receptor.

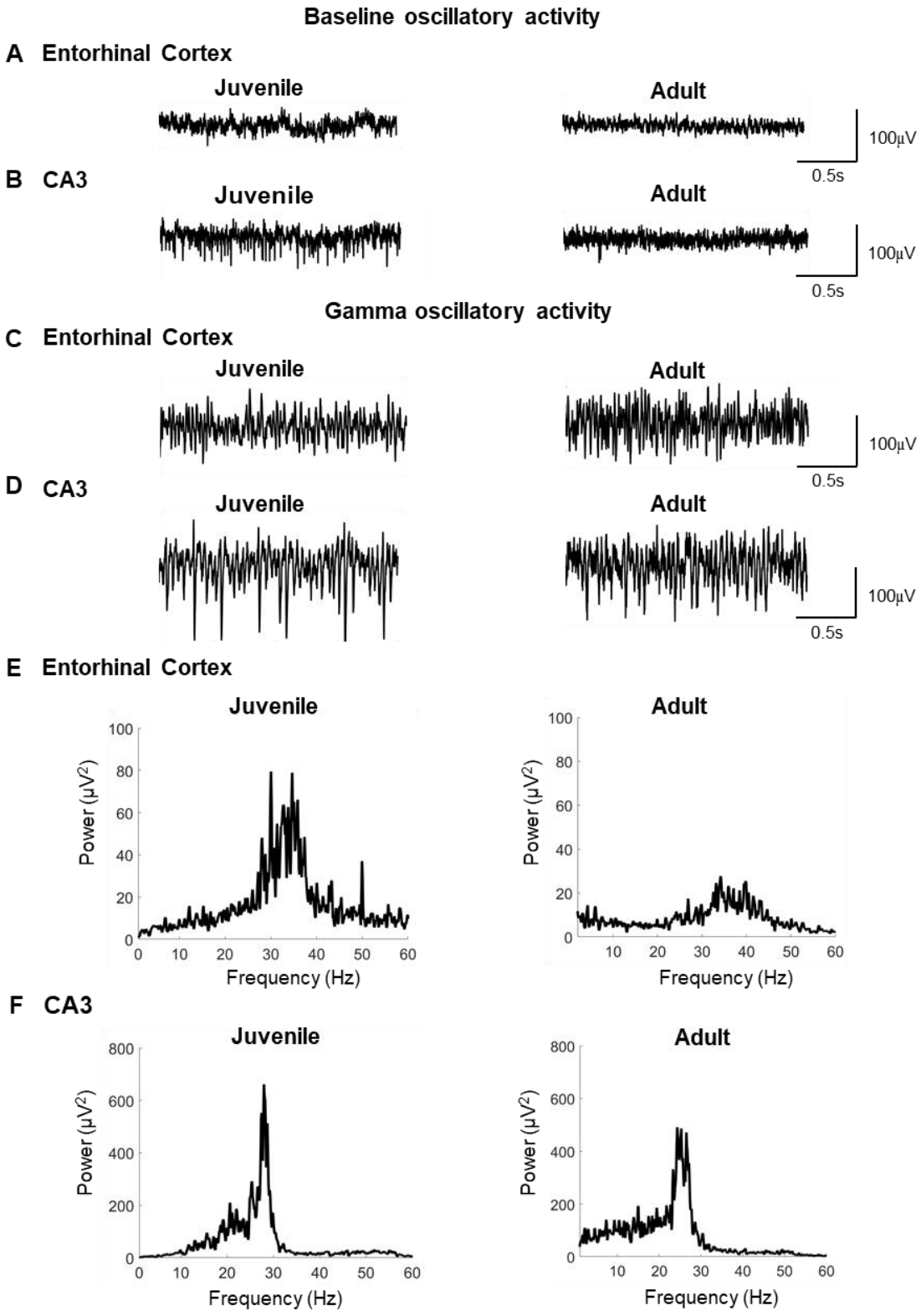


Figure 5.8 Gamma oscillations and power spectrums measured from the entorhinal cortex and CA3 in DARC knockout mice. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) DARC knockout mice. Gamma oscillations were induced by bathing slices in 200 nM kainic acid. **(A–B)** Examples of baseline traces recorded from the entorhinal cortex (A) and CA3 (B). (**C-D**) Examples of gamma oscillation traces recorded from the entorhinal cortex (C) and CA3 (D) in juvenile and adult mice. (**E-F**) Power spectrums generated from gamma oscillations in the entorhinal cortex (E) and CA3 (F) in juvenile and adult mice.

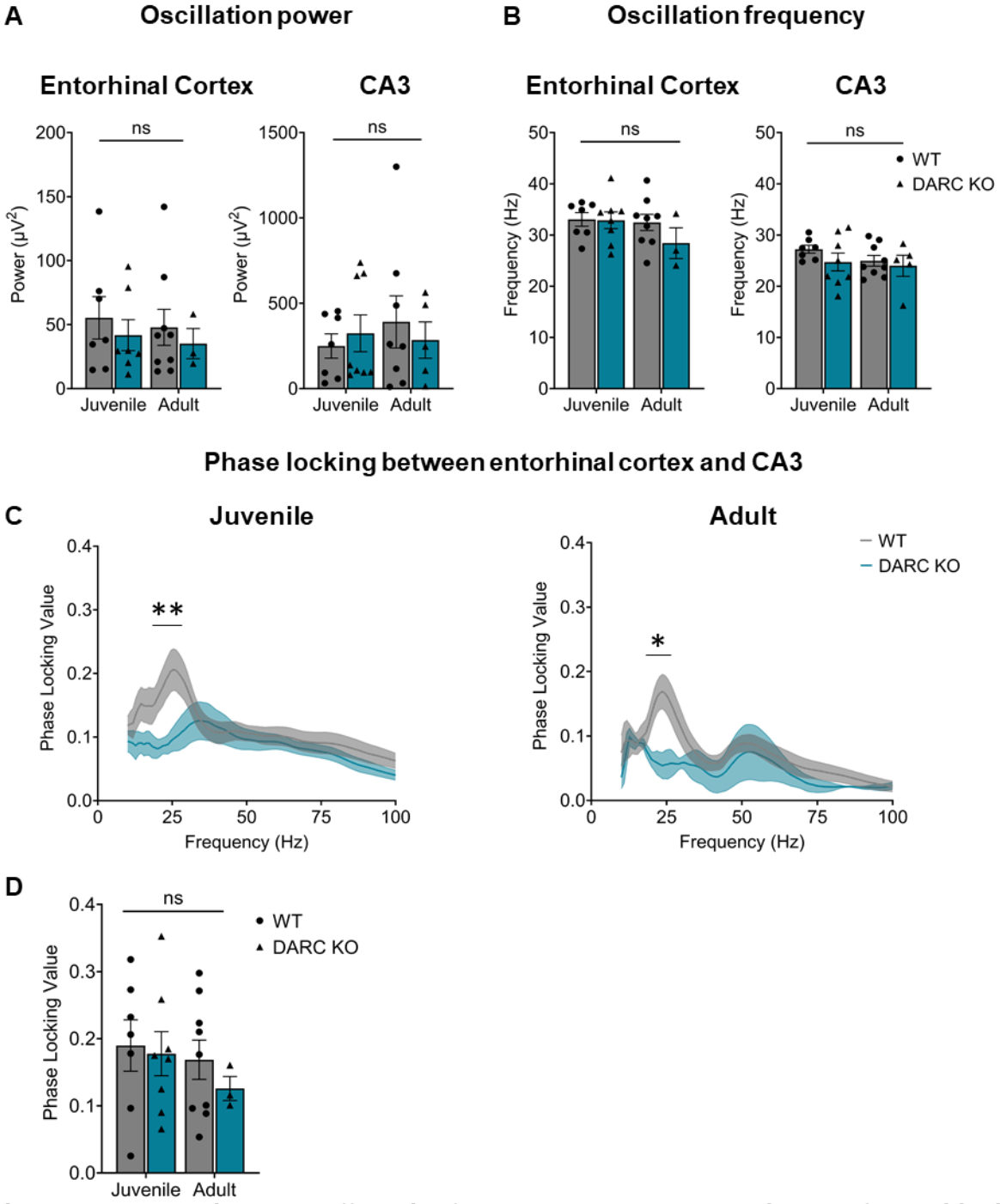


Figure 5.9 DARC does not affect the frequency, power or synchrony of entorhinal cortex and CA3 gamma oscillations. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) DARC knockout mice. Gamma oscillations were induced by bathing slices in 200 nM kainic acid and oscillations were recorded from the entorhinal cortex and CA3 regions in juvenile and adult mice. Results were compared with oscillations gathered from wild-type mice. **(A-B)** Grouped data of gamma oscillation power (A) and frequency (B) measured from the entorhinal cortex and CA3 in juvenile and adult wild-type and DARC knockout mice. **(C)** Entorhinal cortex and CA3 phase locking values as a function of maximum oscillation frequency regions. Significant differences were observed between wild-type and DARC knockout juvenile mice at 21.8-37.3 Hz and between wild-type and DARC knockout adult mice at 22.7-24.6 Hz **(D)** Phase locking values between the entorhinal cortex and CA3 in juvenile and adult wild-type and DARC knockout mice. Bars represent mean ± SEM, * P ≤ 0.05, ** P ≤ 0.0,1 two-way ANOVA with Tukey multiple comparison tests (A,B,D) and Sidak multiple comparisons test (C). N=7 and N=9 mice per group for wild-type juvenile and adult mice respectively. N=8 and N=3 for juvenile and adult DARC knockout mice, respectively (where N number represents 1 hippocampal slice recording per animal).

5.2.4 DARC's role in hippocampal-dependent behaviour

It is well documented that the hippocampus plays an essential role in learning and memory function as hippocampal lesions in rodent models cause severe spatial memory impairment (Jarrard, 1978; Olton, Walker and Gage, 1978; Morris *et al.*, 1982; Sutherland, Whishaw and Kolb, 1983) and impairment of the hormonal stress response in behavioural studies (Dedovic *et al.*, 2009). Communication between the entorhinal cortex and CA3 have been shown to be essential during hippocampal-dependant object learning behaviour (Fernández-Ruiz *et al.*, 2021) and in phase synchronisation between the two subregions is thought to facilitate memory formation, whilst anti-phase synchronisation hinders memory formation (Fell *et al.*, 2001; Fries, 2009). *In vitro* gamma oscillatory experiments described in this thesis, show that in the absence of DARC, in-phase synchronisation of entorhinal cortex and CA3 gamma oscillations are disrupted (Fig 5.9). I next investigated whether DARC knockout mice show changes in hippocampal-dependent behaviour.

It is important to note that the hippocampus runs along a dorsal to ventral axis in rodents, and as the main extrinsic and intrinsic connections are different for dorsal and ventral regions, dorsal and ventral portions are involved in different roles (Ruth, Collier and Routtenberg, 1982; Roberts *et al.*, 1984). The dorsal hippocampus receives sensory information from cortical areas and is associated with cognitive processing such as spatial learning and object recognition (Kim and Fanselow, 1992; Moser, Moser and Andersen, 1993; Moser *et al.*, 1995; McHugh *et al.*, 2011), whilst the ventral hippocampus is involved in emotional processing, such as anxiety (Bannerman *et al.*, 2003; Bannerman *et al.*, 2004) and social behaviour (McHugh *et al.*, 2004; Swanson and Cowan, 1977). The hippocampal slices used for *in vitro* electrophysiology in this thesis were taken from the dorsal plain, therefore I investigated whether the absence of DARC is affecting spatial-learning behaviour.

This was conducted using an established spatial-working memory behavioural test called the Y-maze spontaneous alternation test, which measures a rodent's willingness to explore new environments (Ukai, Shinkai and Kameyama, 1996). Spontaneous alternation is achieved when an animal enters a new arm rather than returning to one visited previously. As rodents typically prefer to investigate new environments, they

usually show a tendency to enter a less recently visited arm (Hughes, 2004; Bak *et al.*, 2017).

Wild-type or DARC knockout juvenile (9-15 weeks old) and adult mice (25-35 weeks old) were placed into the Y-maze and were left to explore for 5 minutes. Rodent movement was recorded, and the behavioural parameters measured were total number of arm entries and percentage alternation, where an alternation represents a mouse entering each 3 arms of the maze consecutively. DARC knockout mice exhibited no differences in spatial working memory compared to wild-type animals (Fig 5.10). No significant differences in total number of arm entries (Fig 5.10 A) or spontaneous alternation (Fig 5.10 B) were identified between wild-type and DARC knockout individuals or between age-cohorts. These data suggest that although intrinsic and post-synaptic properties are disrupted in the absence of DARC, as well as cortical-hippocampal gamma synchrony, spatial-working memory in mice is unaffected. Furthermore, no differences in spatial-working memory were identified in early aging, suggesting that the subtle changes in hippocampal excitability observed in early aging does not affect performance in a Y-maze task.

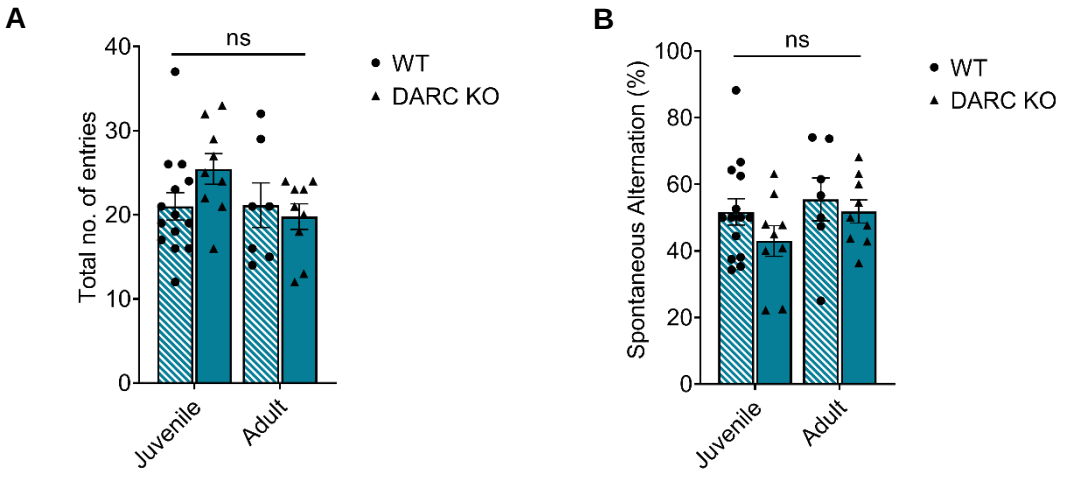


Figure 5.10 DARC knockout mice exhibit no changes in spatial working memory compared to wild-type mice. Spatial working memory of juvenile (9-15 weeks of age) and adult (25-35 weeks of age) wild-type and DARC knockout mice was measured using a Y-maze spontaneous alternation test. Rodent's behaviour was measured for a 5 minute period. **(A)** Grouped data showing the total number of Y-maze arm entries in a 5 minute period. **(B)** Grouped data showing percentage of spontaneous alternation, a measure of rodent's willingness to explore a new environment. Bars represent mean ± SEM, two-way ANOVA with Tukey multiple comparison tests. N=14 and N=7 mice per group for juvenile and adult wild-type mice respectively and N=9 mice for both DARC knockout groups (where N number represents 1 hippocampal slice recording per animal).

I next investigated whether ventral hippocampal-dependent behaviour was affected by DARC or by early aging. The ventral hippocampus is involved in emotional processing, such as anxiety (Bannerman *et al.*, 2003; Bannerman *et al.*, 2004) and social-related behaviour (McHugh *et al.*, 2004; Swanson and Cowan, 1977). This was conducted using an open field behavioural study which assesses rodents general locomotor activity levels, anxiety and willingness to explore an environment (Denenberg, 1969). Juvenile and adult wild-type and DARC knockout mice were placed into an open field arena and their behaviour was recorded for 10 minutes. Several behavioural parameters were measured which included time spent in inner and outer zones of the arena, rearing behaviour, thigmotaxis (wall-hugging behaviour) and total distance travelled. Increased anxiety is related to decreased locomotor activity and rearing behaviour, and increased wall hugging behaviours (Liebsch *et al.*, 1998). Others postulate however, that an increase in rearing behaviour is indicative of increased anxiety instead of increased exploratory behaviour (Costall *et al.*, 1989).

Wild-type and DARC knockout mice spent a significantly greater amount of time in the outer zone of the open arena compared to the inner zone, which occurred regardless of age (Fig 5.11 A). These data suggest that mice are less willing to explore central zones. There were no differences in any anxiety-related parameters between wild-type and DARC knockout mice, nor between juvenile and adult age cohorts (Fig 5.11). These results indicate that early aging does not affect dorsal or ventral hippocampal-related behaviours. It also shows that although DARC modulates dentate gyrus cellular properties, hippocampal-dependent behaviour is not affected.

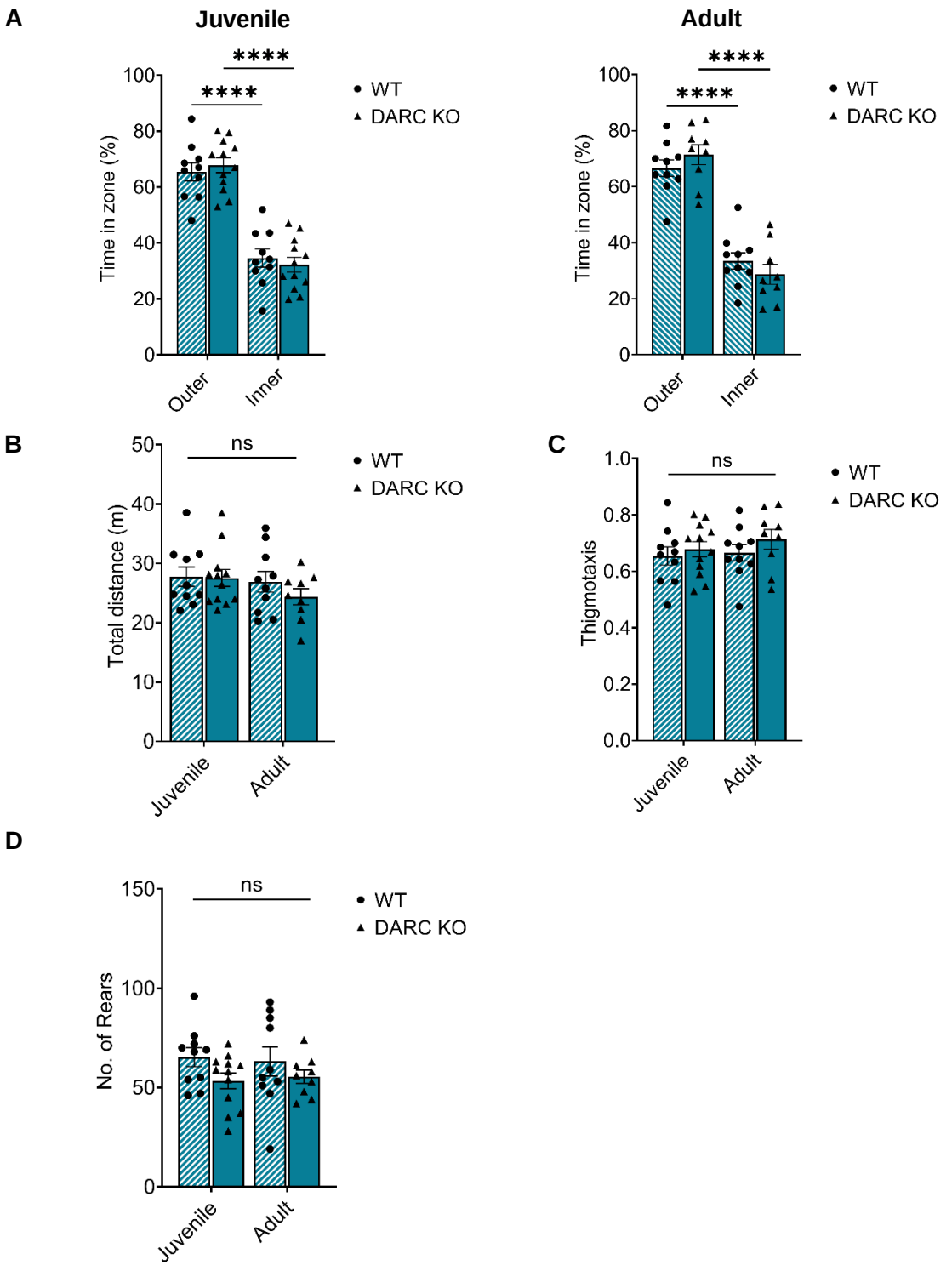


Figure 5.11 DARC knockout mice exhibit no changes in exploratory and anxiety related behaviours compared to wild-type mice. Anxiety-related behaviour and exploratory behaviour was measured using an open-field arena test in juvenile (9-15 weeks of age) and adult (25-35 weeks of age) wild-type and DARC knockout mice. Behaviour was measured for a 10 minute period. **(A)** Grouped data showing average proportion of time spent in the outer zone compared to the inner zone during a 10 minute period in the open arena. **(B)** Total distance travelled in a 10 minute period. **(C)** Anxiety-related wall-hugging behaviour, thigmotaxis, measured during a 10 minute period. **(D)** Number of times rodent rears onto hind legs. Bars represent mean ± SEM, two-way ANOVA with Tukey multiple comparison tests. N=10 and N=12 mice per group for juvenile and adult wild-type mice respectively and N=9 mice for both DARC knockout groups (where N number represents 1 hippocampal slice recording per animal).

5.2.5 DARC's role in mediating CCL2-dependent hippocampal excitability

DARC is thought to play a subtle role in mediating inflammation (Pruenster *et al.*, 2009) and can bind to several proinflammatory chemokines including CCL2. DARC can affect circulating levels of CCL2 (Fukuma *et al.*, 2003; Jilma-Stohlawetz *et al.*, 2001) and shuttles pro-inflammatory chemokines such as CCL2 and CCL5 across the blood brain barrier (Minten *et al.*, 2014). CCL2 and its cognate receptor CCR2 are located on hippocampal neurons (Banisadr *et al.*, 2005a) and can directly affect dentate gyrus (Fig 4.1) and CA3 excitability (Zhou *et al.*, 2011). DARC expression is also localised to neurons (Fig 5.1) yet its role in the regulation of chemokine activity in the brain has not been studied. I investigated whether DARC is mediating CCL2-dependent changes in dentate gyrus excitability.

This was performed using the same technique as described in 4.2.1. Hippocampal slices were prepared from juvenile (9-15 weeks old) and adult (25-35 weeks old) DARC knockout mice. Control stimulation responses were generated by perforant pathway stimulation in slices bathed in circulating ACSF, before a second stimulus response curve was generated after slices had incubated in 7 nM of circulating CCL2. CCL2 significantly increased field EPSPs (fEPSPs) in the supra-pyramidal dentate gyrus in juvenile and adult DARC knockout mice, and in adult mice in the infra-pyramidal subregion. There was no change in infra-pyramidal fEPSPs in juvenile mice (supra-pyramidal juvenile mice: control, 0.48 ± 0.01 vs +CCL2 1.11 ± 0.2, P=0.0014, adult mice: control, 0.48 ± 0.01 vs +CCL2, 1.01 ± 0.23, P=0.0301. Infra-pyramidal juvenile mice: control, 0.52 ± 0.02 vs +CCL2, 1.23 ± 0.15, ns, adult mice: control, 0.53 ± 0.01 vs +CCL2, 2.29 ± 0.66, P=0.0043, Fig 5.12 C).

Although CCL2 appears to have a somewhat greater effect on dentate gyrus excitability in juvenile wild-type mice compared to juvenile DARC knockout mice (Fig 5.13), these effects were not significant (data not shown). CCL2 significantly increases dentate gyrus excitability with and without the presence of DARC, which indicates that CCL2-mediated increase in hippocampal excitability cannot be attributed to DARC function.

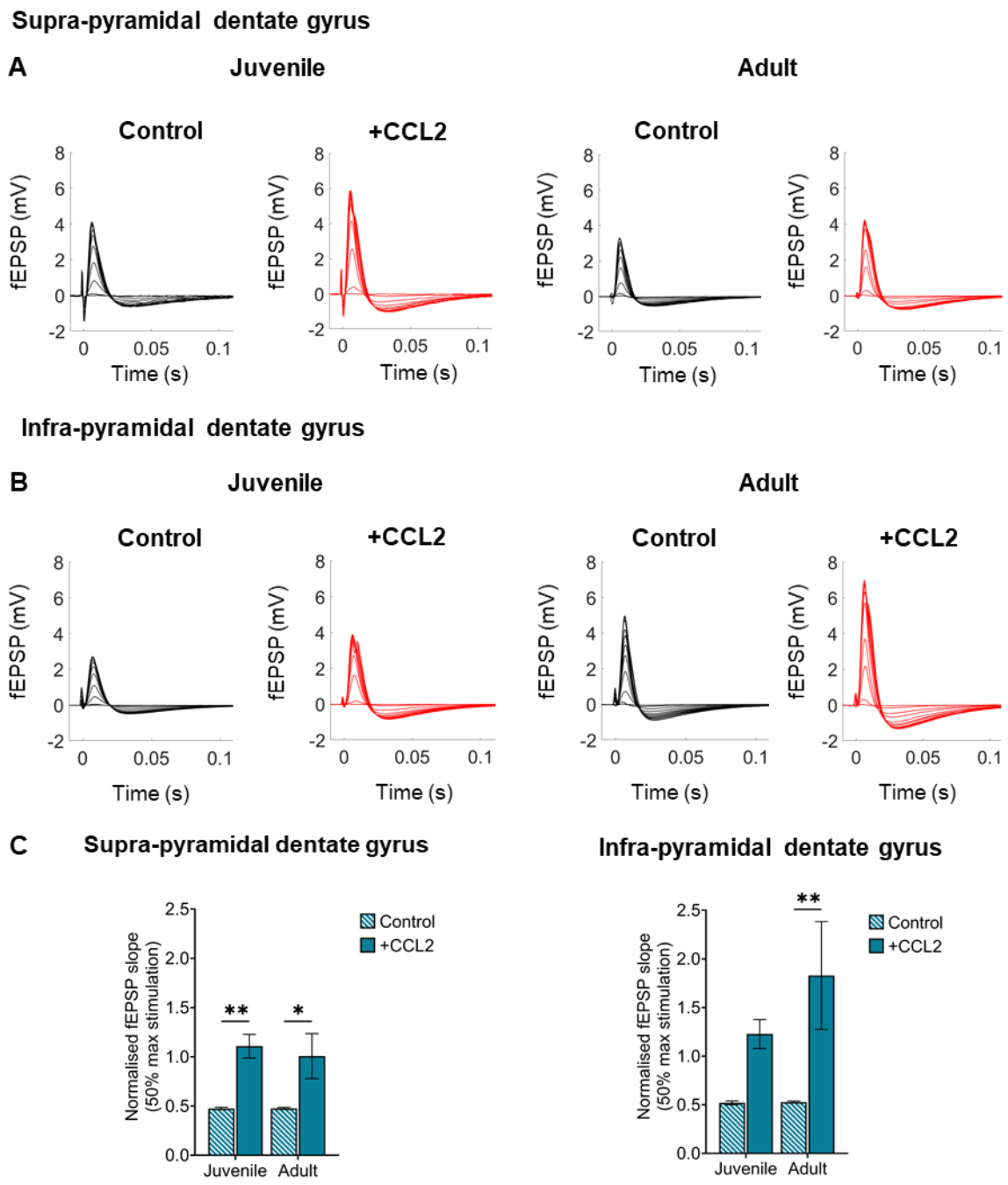


Figure 5.12 CCL2 increases hippocampal excitability in DARC knockout mice. Hippocampal slices were prepared from either juvenile (9-15 weeks of age) or adult (25-35 weeks of age) DARC knockout mice. The perforant path was activated by electrical stimulation of layer II of the entorhinal cortex. Control recordings were taken from slices incubated with ACSF. Slices were then incubated with 7 nM of CCL2 for 1 hour and a second recording taken. **(A–B)** Representative field EPSPs (fEPSPs) recorded from the supra-pyramidal (A) or infra-pyramidal blade (B) of the dentate gyrus in juvenile or adult mice, from control slices or with 7 nM CCL2. The entorhinal cortex was stimulated at time 0. fEPSPs represent the average response of 10 electrical stimulations (1 Hz) for each intensity (5V–100V). **(C)** Group data showing the effect of CCL2 on fEPSP responses in the supra-pyramidal or infra-pyramidal dentate gyrus in DARC knockout mice. Data is normalised to fEPSPs at 50% maximum stimulation intensity. Bars represent mean ± SEM. * P ≤ 0.05, ** P ≤ 0.01 two-way ANOVA with Tukey multiple comparison tests. N=11 juvenile mice and N=8 adult mice for each group (where N number represents 1 hippocampal slice recording per animal).

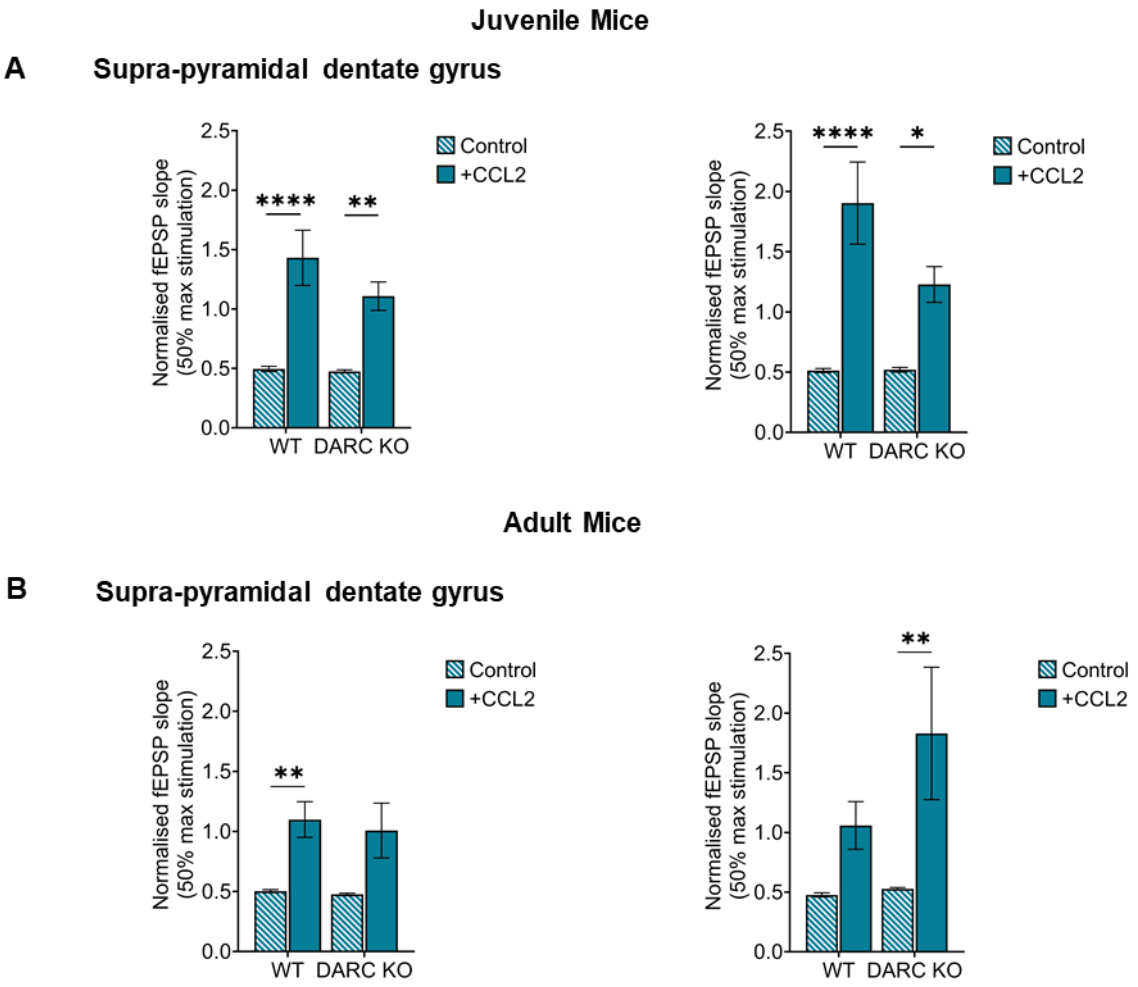


Figure 5.13 DARC does not mediate CCL2's effect on increasing hippocampal excitability. (A–B) Group data of fEPSPs showing the effect of CCL2 on fEPSP responses in juvenile (A) and adult (B) wild-type and DARC knockout mice. Data is represented at 50% maximum stimulation intensity recorded from the supra-pyramidal and infra-pyramidal dentate gyrus. Bars represent mean ± SEM. * P ≤ 0.05, ** P ≤ 0.01, ****P<0.0001 two-way ANOVA with Tukey multiple comparison tests. N=11 and N=10 wild-type juvenile and adult mice respectively and N=11 and N=8 DARC knockout juvenile and adult mice respectively. (N number represents 1 hippocampal slice recording per animal).

5.2.6 CCL2's effect on microglia in the absence of DARC

In this chapter I have shown that DARC knockout does not affect CCL2-dependent increase in hippocampal excitability (Fig 5.12 and 5.13). It is well reported that DARC is involved in regulating CCL2 concentration in the blood (Fukuma *et al.*, 2003; Jilma-Stohlawetz *et al.*, 2001), and aids chemokine trafficking during inflammation (Minten *et al.*, 2014), however its role in chemokine regulation in the brain remains elusive. I have shown that DARC does not regulate CCL2-mediated hippocampal excitability, however it is possible that the receptor is regulating other downstream CCL2-dependant mechanisms.

CCL2 is able to influence microglia activity and morphology, as neuron derived CCL2 can induce the production of new microglia and increase microglia proliferation (Hinojosa *et al.*, 2011). I have shown that CCL2 increases microglia branch length in wild-type juvenile mice (Fig 4.7), but no other significant changes in microglia morphology or proliferation were observed in early aging (Fig 4.7 – 4.10). I next investigated whether DARC could be playing a role in microglial activation by assessing basal and CCL2-dependent changes in microglia morphology in DARC knockout mice.

This was conducted by examining juvenile (9-15 weeks old) and adult (25-35 weeks old) DARC knockout hippocampal slices that were used for the *in vitro* electrophysiology experiment described in 5.2.5, that were either incubated in circulating ACSF or 7nM CCL2 for 1 hour. Slices were collected, fixed, cryoprotected and re-sectioned before immuno-fluorescence immunohistochemistry was conducted on re-sectioned brain slices, staining for the microglia marker ionised calcium binding adapter 1 (Iba1), the mature neuronal marker Neu-N, and the nuclear stain Hoechst. The microglia staining was captured using confocal microscopy. Microglia number was generated by counting the individual cells from confocal images at a x20 objective. Microglia morphology parameters were calculated using Sholl analysis on confocal images captured at a x63 objective. A higher magnification was used to clearly identify microglia projections.

CCL2 incubation reduced microglia branch length in adult DARC knockout mice (control, 36.73 ± 2.450µM vs +CCL2, 28.90 ± 3.653µM, P=0.0012, Fig 5.15) however no changes in microglia morphology were observed in juvenile mice (Fig 5.14).

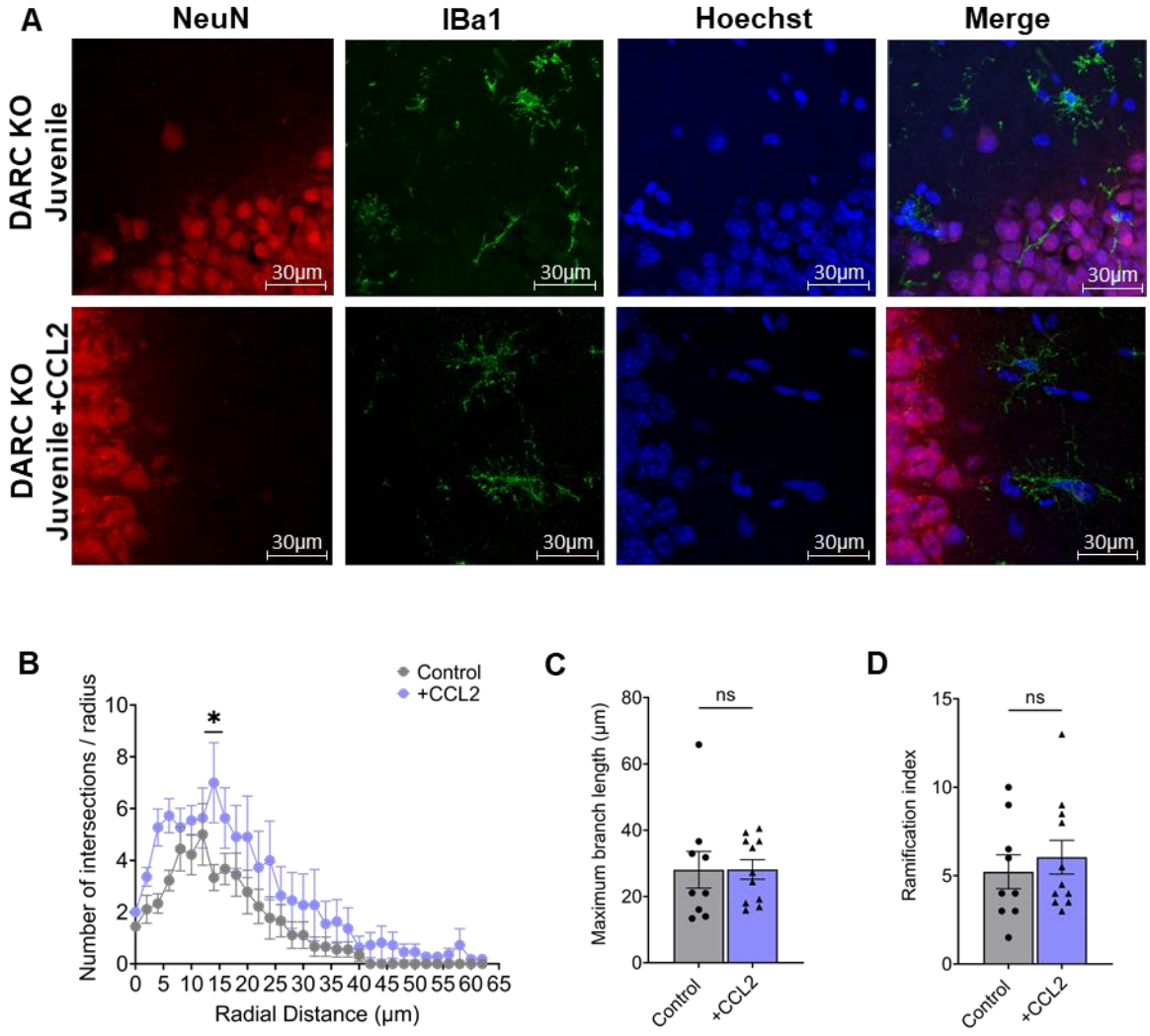


Figure 5.14 CCL2 has no effect on microglia morphology in juvenile DARC knockout mice. DARC knockout hippocampal slices from juvenile (9-15 week old) mice used for electrophysiology were collected, fixed in PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Slices were collected that had been bathed in ACSF and in circulating 7 nM CCL2. Free-floating immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. The primary antibodies IBa1 microglia stain, NeuN mature neuronal stain and Hoechst nuclear stain were used. (**A**) Representative maximum intensity projection images of immuno-staining collected using a confocal microscope at a x63 objective. Single channel Iba1, NeuN and nuclear stain are presented along with a merge of all three channels for juvenile mice treated with control or CCL2. (**B-D**) Scholl analysis of captured images indicating the number of microglia branching intersections radial distance (B), maximum branch length (C) and ramification index (D). Bars represent mean ± SEM, * P ≤ 0.05, Mann-Whitney test. N=9 and N=11 cells for control and +CCL2 groups respectively (N represents cell number measured across 3 hippocampal slices from 3 individual mice).

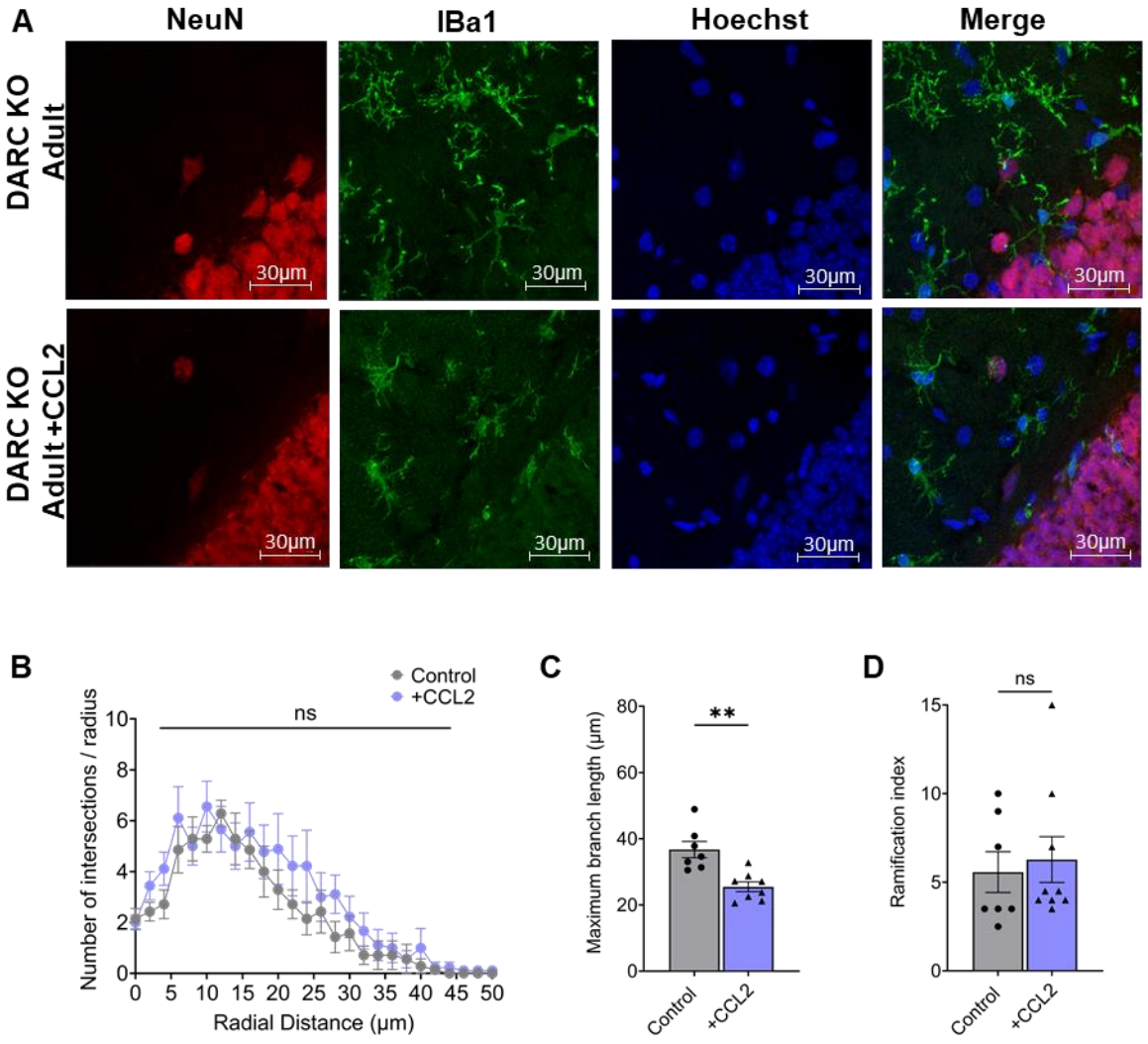


Figure 5.15 CCL2 decreases microglia branch length in adult DARC knockout mice. DARC knockout hippocampal slices from juvenile (25-35 week old) mice used for electrophysiology were fixed, stored in PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Slices were collected that had been bathed in ACSF and in circulating 7 nM CCL2. Free-floating immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. The primary antibodies IBa1 microglia stain, NeuN mature neuronal stain and Hoechst nuclear stain were used. (**A**) Representative maximum intensity projection images of immuno-staining collected using a confocal microscope at a x63 objective. Single channel Iba1, NeuN and nuclear stain are presented along with a merge of all three channels for juvenile mice treated with control or CCL2. (**B-D**) Scholl analysis of captured images indicating the number of microglia branching intersections radial distance (B), maximum branch length (C) and ramification index (D). Bars represent mean ± SEM, ** $P \leq 0.01$, Mann-Whitney test. N=7 and N=9 cells for control and +CCL2 groups respectively (N represents cell number measured across 3 hippocampal slices from 3 individual mice).

I next investigated whether DARC is affecting microglia cell number. In wild-type animals, I have identified that there is no difference in microglia cell number after CCL2 incubation or in early aging (Fig 4.10). However, significant differences in microglia morphology and proliferation were observed in DARC knockout mice. In DARC knockout adult mice, microglia number was significantly reduced after CCL2 addition in the cortex (control, 21.25 ± 1.109 vs +CCL2, 12.75 ± 2.529, P=0.0148), yet no difference was observed in juvenile mice, (Fig 5.16 B). In the hilus, microglia number was unaffected by CCL2 incubation. Notably, adult mice had a greater microglia cell count compared to juveniles (Control: juvenile, 10.67 ± 0.385 vs adult, 18.92 ± 1.805, P=0.0047. +CCL2: juvenile, 9.56 ± 0.988 vs adult, 17.5 ± 1.04, P=0.0059 Fig 5.14 C), which was not observed in wild-type mice (Fig 4.10). These data indicate that in the absence of DARC, microglia proliferation occurs in early aging in the hilus. These data suggest a role for DARC in controlling cortical microglia proliferation in adult mice.

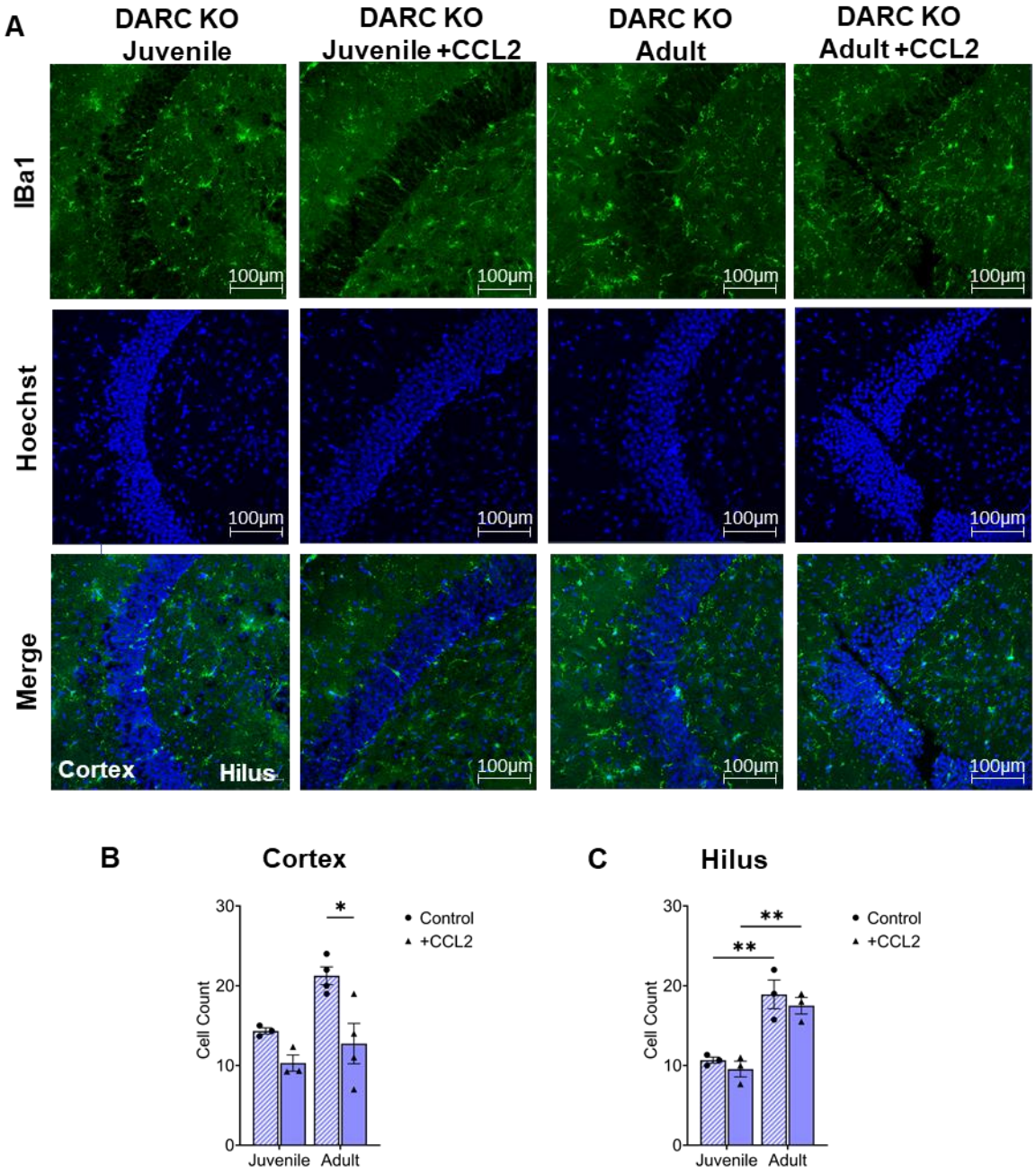


Figure 5.16 CCL2 decreases cortical microglia number, and adult DARC knockout mice have a higher hilus microglia count compared to juveniles. Hippocampal slices from juvenile (9-15 weeks old) and adult (25-35 weeks old) DARC knockout mice used for electrophysiology were collected, stored in PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Slices were collected that had been bathed in ACSF and in circulating 7 nM CCL2. Free-floating immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. The primary antibodies IBa1 microglia stain and Hoechst nuclear stain were used. (**A**) Representative maximum intensity projection images of immuno-staining collected using a confocal microscope at a x20 objective. Single channel Iba1 and nuclear stain are presented along with a merge of both channels for mice treated with ACSF or CCL2. (**B-C**) Microglia cell count from the cortical area (B) and hilus (C) of the dentate gyrus. Bars represent mean ± SEM, two-way ANOVA with Tukey multiple comparison tests. N=3 mice per group and N=4 mice for cortical adult cell counts, with 4 hippocampal slices measured and averaged per mouse.

5.3 Discussion

The Duffy antigen receptor for chemokines (DARC) is a non-signalling transmembrane spanning receptor (Lee *et al.*, 2003), that is expressed on red blood cells and the postcapillary endothelium (Segerer *et al.*, 2000) in a number of peripheral organs including lymph nodes (Kashiwazaki et al., 2003), the lung (Lee et al., 2003), and the kidney (Hadley and Peiper, 1997). DARC binds to pro-inflammatory chemokines of the CXC and CC families, including CXCL1, CCR5 and CCR2 (Gardner *et al.*, 2004) and acts to buffer plasma chemokine levels, reduces levels of circulating pro-inflammatory chemokines which dampens systemic leukocyte activation (Dawson *et al.*, 2000; Zarbock *et al.*, 2010). More recently, DARC expression has been detected at the blood–brain barrier (BBB) where it acts to aid the translocation of pro-inflammatory chemokines CCL5 and CCL2 across the blood-brain barrier (BBB). Translocation is increased during systemic inflammation and is markedly reduced in DARC knockout mice. (Minten *et al.*, 2014). DARC expression has also been identified in the brain, localised almost exclusively in neurons (Schaum *et al.*, 2018), however its prevalence has only been confirmed on Purkinje neurons in the cerebellum (Horuk *et al.*, 1997; Horuk *et al.*, 1996). There has been no further investigation into what role DARC may play in neurons, its expression in other brain regions, nor into how its expression may change during aging. Therefore, in this chapter I aimed to investigate if DARC played a role in mediating hippocampal excitability using a homozygous DARC knockout mouse line.

5.3.1 DARC mediates age-related hippocampal excitability through changes in dentate gyrus extrinsic properties

I have previously shown in chapter 3 that hippocampal excitability increases in early aging. This increase was more marked in the supra-pyramidal dentate gyrus compared to the infra-pyramidal. Furthermore, supra-pyramidal dentate gyrus neuron's intrinsic membrane properties also changed in early aging whereas no changes were seen in infra-pyramidal neurons. The increase in dentate gyrus excitability in early aging was eliminated in DARC knockout mice, suggesting that DARC-dependent cellular events contribute to age-dependent changes in hippocampal excitability. Measuring intrinsic properties of supra-pyramidal and infra-pyramidal granule neurons in DARC knockout mice revealed that there was a significant reduction in EPSP amplitude in DARC knockout adult supra-pyramidal granule neurons, generated by stimulation of perforant pathway fibres. As changes in EPSP amplitude are associated with post-synaptic

changes (Van der Kloot, 1991), this indicates that DARC may modulate supra-pyramidal granule activity through post-synaptic mechanisms, although it is not clear whether this is caused by changes in post-synaptic sensitivity, quantity of neurotransmitter released or changes in driving force for ions conducting the EPSPs (Van der Kloot, 1991). It is important to note, that a caveat of this experiment is that EPSP amplitude is dependent on the resting membrane potential. Any significant changes in resting membrane potential, will affect EPSP closeness to the reversal potential. Therefore, it is not conclusive that DARC modulates dentate gyrus granule neuron activity through post synaptic mechanisms.

Adult supra-pyramidal granule neurons had a depolarised resting membrane potential in DARC knockout mice, suggesting that the hyperpolarisation of resting membrane potential in early aging observed in wild-type mice, is also mediated by DARC. Additionally, adult infra-pyramidal granule neurons in DARC knockout mice had increased membrane resistance, although this appears to not elicit excitability changes extracellularly. It is not clear from these experiments whether DARC's mediation of hippocampal excitability in early aging is via excitatory post-synaptic activity or through changes in inhibition. This could be assessed by measuring AMPA and NMDA receptor currents in patch clamp experiments in DARC null neurons to assess if DARC affects postsynaptic currents. Spontaneous miniature IPSCs and EPSCs could be measured to examine whether DARC knockout causes an imbalance between inhibition and excitation in the dentate gyrus.

Little is known about DARCs expression in the hippocampus, whether its expression is hippocampal wide or subregion specific, or whether expression levels change in early aging. It is also unknown if DARC is regulating neuronal excitability in other hippocampal subregions such as the CA3 and CA1. This could be investigated by measuring excitability of CA3 and CA1 neurons using the same electrophysiological techniques described in 5.2.1 and 5.2.2, but instead stimulating mossy fibres or Schaffer collateral fibres, respectively. Furthermore, it is not clear whether a global DARC deficiency results in the upregulation of other pro-inflammatory chemokine receptors. DARC expressed on endothelial cells is reported to promote chronic inflammation by reducing chemokine concentrations in inflamed tissue by internalising chemokines and creating a gradient that increases monocyte extravasation (Bonecchi and Graham, 2016). However, it is not known whether DARC conducts a similar activity on neurons. It is important that future

work examines the expression patterns of DARC in the hippocampus to understand how receptor localisation affects hippocampal activity.

5.3.2 Microglia cell number increases in early aging in DARC knockout mice

Staining DARC knockout hippocampal slices for the Iba1 microglia marker revealed that microglia number in the hilus of the dentate gyrus increases in early aging, but no changes to microglia morphology are observed. This suggests that DARC regulates microglia proliferation during early aging. Microglia proliferation is known to increase in response to inflammation and upregulation of pro-inflammatory chemokines (He *et al.*, 2016; Zhang and De Koninck, 2006; Tian *et al.*, 2017), and DARC has been described to remove surplus chemokines from circulation as high levels of chemokines can induce the desensitization of their cognate receptors (Darbonne *et al.*, 1991). Therefore, it is possible that chemokine levels in the hippocampus of DARC knockout mice increase during early aging, in turn promoting microglia proliferation. To investigate this further, chemokine profiling in DARC knockout and wild-type mice would need to be conducted, preferably in the blood, brain, and cerebral spinal fluid, to detect if and what chemokines are upregulated in the absence of DARC in the periphery and CNS. The upregulation of a few pro-inflammatory cytokines and chemokines have been identified in DARC knockout mice, including increased basal circulating CCL2 and IL-8, which increased systemic leukocyte activation (Dawson *et al.*, 2000). Additionally, CXCL1 and CXCL2 are upregulated in the plasma and lung of DARC knockout mice after LPS injection (Reutershan *et al.*, 2009). Both human and rodent studies show that DARC can sustain inflammatory chemokines levels on erythrocytes and in plasma (Jilma-Stohlawetz *et al.*, 2001; Fukuma *et al.*, 2003).

Conversely, circulatory chemokine levels are also reported to decrease in the absence of DARC, as DARC is hypothesised to also act as a chemokine buffer by increasing their systemic availability during inflammation (Lee *et al.*, 2003). Basal plasma CCL2 levels are one-third lower in DARC knockout mice than in control wild-type animals, and when CCL11 or CXCL1 were administered intravenously, these chemokines disappeared more rapidly from the plasma of DARC knockout mice (Fukuma *et al.*, 2003). Furthermore, DARC negative humans were noted to have significantly lower basal CCL2

levels than Duffy-positives (Jilma-Stohlawetz *et al.*, 2001). It is important to note, that in all references, experiments were conducted on mice aged between 4-9 weeks old, and human subjects had an average age of 28. I noted a significant increase in microglia number in DARC knockout adult mice compared to DARC null juveniles, suggesting that DARC affects hippocampal inflammatory responses in early aging. Future experiments would aim to detect levels of DARC, and their expression patterns in my juvenile and adult age groups.

5.3.3 DARC affects phase locking in cortical-hippocampal gamma oscillations but not hippocampal associated behaviour

As DARC plays a role in regulating perforant path excitability, I next investigated whether DARC deficiency also affected cortical-hippocampal gamma network activity. No significant changes in the frequency or amplitude of gamma oscillations in the entorhinal cortex and CA3 was identified. However, phase locking between the entorhinal cortex and CA3 was significantly different between wild-type and DARC knockout juvenile and adult mice at the gamma frequency (at 21.8-27.28 Hz frequencies, and 22.73-24.55 Hz frequencies, respectively). These results suggest that DARC is responsible in mediating phase locking between the entorhinal cortex and CA3 at gamma frequencies.

I also investigated whether elimination of DARC was affecting hippocampal-dependent behaviour. Different hippocampal regions along its dorsal-ventral axis control different behaviours due to differences in cellular and circuit properties (Risold and Swanson, 1997). The dorsal hippocampus, the region explored in this thesis, is involved in spatial-learning and episodic memory function, whilst the ventral hippocampus is associated with anxiety and reward processing behaviours (Bannerman *et al.*, 2002; Moser and Moser, 1998). Both behavioural parameters were investigated in juvenile and adult DARC knockout mice to determine whether DARC affects hippocampal-dependent function in early aging. A Y-maze spontaneous alternation test was carried out to examine spatial-learning behaviour, whilst an open-field maze was used to measure anxiety and explorative behaviour. I observed no behavioural differences between wild-type and DARC knockout mice in the Y-maze or open-field study, in either age-cohort, suggesting that although DARC expression affects hippocampal activity in early aging, this does not manifest in behavioural differences in the paradigms tested in this thesis.

In contrast to my findings, behavioural differences have been reported in previous work (Schneider *et al.*, 2014) that used 3-5 month old DARC knockout mice (Dawson *et al.*, 2000) from the same genetic background as used in this theses. Schneider et al (2014) showed that DARC knockout mice express reduced spatial-navigation and increased anxiety-related behaviour compared to wild-type littermate controls (Schneider *et al.*, 2014) using the Morris-water maze task for spatial-learning memory and an elevated plus maze to assess anxiety. They also observed that DARC knockout mice had impaired balance in a rotary rode study (Schneider *et al.*, 2014). The behavioural phenotypes were attributed to DARC deficiency and are consistent with other publications showing cerebellum expression of DARC (Horuk *et al.*, 1996; Horuk *et al.*, 1997).

Different behavioural studies of cognition explore different aspects of hippocampal-dependent behaviour, and although the Y-maze spontaneous alternation test and open-field test are routinely used to explore spatial-navigation and anxiety behaviour, measuring cognition using the Morris-water maze and elevated plus maze utilises the natural behaviour of rodents (Lister, 1987). This could explain why I did not observe a behavioural phenotype. Additionally, variations in experiment design and test duration time could results in behaviour variability (Ruan and Yao, 2020), and differences in rodent husbandry can also affect outcomes (Sare, Lemons and Smith, 2021).

5.3.4 CCL2 increasing hippocampal excitability is not mediated by DARC

Although CCL2 binds to DARC with high affinity (Hansell, Hurson and Nibbs, 2011), and DARC is involved in chemokine translocation across the BBB (Minten *et al.*, 2014), *in vitro* electrophysiology experiments revealed that CCL2 significantly increases dentate gyrus excitability with and without the presence of DARC, indicating that CCL2-mediated increase in hippocampal excitability cannot be attributed to DARC function. It is unknown whether CCL2 expression in the hippocampus is altered in DARC knockout mice, although there is conflicting evidence as to whether CCL2 expression is upregulated or reduced in blood is the absence of DARC (Dawson *et al.*, 2000; Fukuma *et al.*, 2003).

Notably, microglia branch length was significantly reduced after CCL2 incubation in adult DARC knockout mice, which was not observed in wild-type mice. Branch shortening is associated with microglia de-ramification after activation (Fernandez-Arjona *et al.*, 2017;

Davis, Foster and Thomas, 1994), suggesting that DARC regulates CCL2-acitvation of hippocampal microglia. This effect was not observed in juvenile mice, therefore microglia sensitivity to CCL2-mediated activation is increased during early aging. DARC's sink function could be regulating these changes by removing excess CCL2 from the blood or from tissues (Darbonne *et al.*, 1991). Additionally, CCL2 addition resulted in a reduction in microglia cell number in the cortical side of the dentate gyrus but not the hilus, which proposes that CCL2 induces microglia migration out of the cortical area.

5.4 Future work

This chapter describes a novel role for the atypical chemokine receptor DARC in regulating hippocampal excitability and microglial activation during early aging. Future work would be aimed at investigating the mechanisms underlying DARC-dependent regulation of hippocampal excitability. The amplitude and frequency of AMPA and NMDA receptor currents could be assessed in patch clamp experiments in DARC null neurons to assess if DARC affects postsynaptic currents. Spontaneous miniature IPSCs and EPSCs could be measured to examine whether DARC knockout causes an imbalance between inhibition and excitation in the dentate gyrus. Measuring synaptic number in the dentate gyrus could help to uncover whether synaptic number is contributing to the different excitability measurements. This could be achieved by immuno-fluorescence staining of pre- and post-synaptic markers in hippocampal tissue. Additionally, the experiments reported in this chapter conclude that DARC modulates excitability in the dentate gyrus, and it is not known whether DARCs effects are subregional or hippocampal wide. Measuring excitability of neurons in the CA3 and CA1 in DARC knockout mice could provide this information.

To fully understand DARCs role in modulating age-dependent hippocampal activity, DARC expression level and its localisation in the hippocampus is crucial. I have previously attempted to measure hippocampal DARC expression by immunohistochemical staining of slices with an anti-DARC knockout antibody. However, the anti-DARC antibody bound non-specifically, indicating positive DARC staining in DARC knockout brain tissue (Figure 1, appendix). This was hypothesised to be caused by the DARC antibody binding non-specifically to other ACKR1 receptors such as D6 (ACKR2). An alternative to immunohistochemistry staining of DARC would be to isolate different brain regions and assess relative DARC mRNA levels. Although this would allow

for the quantification of DARC in different brain regions, expression localisation would not be detected.

The chemokine or chemokine receptor profile of the DARC knockout mouse model has not been investigated in a systematic and comprehensive manner. This information is essential to understand whether DARC directly regulates hippocampal function, or instead via regulating chemokine levels. Additionally, DARC can oligomerise with other chemokine receptors such as CCR5 to influence downstream signalling (Chakera *et al.*, 2008). A cytokine array such as the Milliplex MAP mouse Cytokine/Chemokine Magnetic Bead Panel Kit (Covaris, USA), which allows for simultaneous quantification of many chemokines and chemokine receptor analytes in samples of tissue lysate and serum could be used to map chemokines and receptor expression throughout different brain regions, data which is currently missing from the field.

5.5 Summary

The results in this chapter describe a novel role for the atypical chemokine receptor DARC in regulating hippocampal excitability and microglia proliferation during early aging, which was investigated using DARC homozygous knockout mice. I also report that the assumed DARC expression in dentate gyrus granule neurons regulates intrinsic membrane properties including resting membrane potential and membrane resistance, which change when aging from juvenile to adulthood. These are the first reported effects of DARC deficiency on brain cells.

No significant changes in hippocampal gamma oscillations or hippocampal-dependent behaviour were identified in DARC knockout mice. These data suggest that DARC plays no regulatory role in the gamma hippocampal network nor in the hippocampal-dependent behavioural parameters investigated in this thesis.

Future work could involve measuring DARC expression level and its localisation in the hippocampus using ELISAs or chemokine mapping kits. Furthermore, measuring the excitability of CA3 and CA1 neurons using the *in vitro* electrophysiological techniques described in this chapter, could help to identify whether DARCs effects are subregional or hippocampal wide.

Chapter 6 – General discussion and future experiments

6.1 Overview

Like all other organs, the brain undergoes changes during the lifespan that impact on information processing and cognitive ability. During the life course different molecular and cellular changes occur across all brain cell types to alter neuronal connectivity and neural circuits underlying behaviour. Research on age-dependent cognitive decline typically compares young and old cohorts in humans, and rodent studies used to understand aging are largely focussed on similar comparisons. To better understand biological processes underlying aging, longitudinal studies on a range of ages are required. In this thesis I examined how hippocampal excitability changes during early aging. As aging is associated with an increase in systemic and brain inflammatory chemokines, which can influence the function of all brain cell types, I investigated how acute chemokine treatment and deficiency of an atypical chemokine receptor affect hippocampal excitability during early aging.

Here I summarise my main findings, discuss their broad implications and consider how future work could further improve understanding of hippocampal age-dependent changes and the role of chemokines and their receptors in regulating hippocampal function.

6.2 Hippocampal excitability is increased in early aging

It is well documented that the hippocampus is one of the brain regions that is greatly affected during aging, with reductions in synaptic excitability, long-term potentiation (Barnes, 1979) and disruptions in hippocampal oscillations reported in rodent and human models (Vreugdenhil and Toescu, 2005; Lu *et al.*, 2011; Murty *et al.*, 2020). These functional changes are all associated with reduced learning and memory performance in rodent (Gage *et al.*, 1984; Mizumori, Lavoie and Kalyani, 1996; Shen *et al.*, 1997), primate (Rapp, Kansky and Roberts, 1997) and human behavioural models (Newman and Kaszniak, 2000; Moffat, Zonderman and Resnick, 2001), highlighting that cognition is greatly reduced in normal aging and is associated with the onset of neurodegenerative diseases such as Alzheimer's disease (Boyle *et al.*, 2013; Ritchie *et al.*, 2018).

Most data investigating hippocampal function during aging focuses predominantly on comparing young (6 weeks – 4 months of age in rodents and 9-25 years old in humans) with aged (14+ months of age in mice, and 60+ for human subjects) cohorts, which is

deemed as late aging (Radulescu *et al.*, 2021). However, aging is defined as the progressive deterioration of physiological function with increasing age (Galloway, 1993), yet there is very little literature examining the effects of early aging and whether physiological aging is also progressive. This is an important topic as understanding the very earliest stages of senescence may allow for the development of targeted approaches that can slow the negative impact of aging or even rejuvenate function (Wyss-Coray, 2016; Castellano *et al.*, 2017). More recently there has been study into how hippocampal function is affected in middle age. Hippocampal-dependent spatial memory in humans has been shown to gradually decline between young (18-25 year olds), middle age (40-55 year olds) and the elderly (60 + years old) (Williams *et al.*, 2019) which is associated with clinical onset of Alzheimer's Disease (Ritchie *et al.*, 2018). Additionally, neural circuitry has been documented to be become disrupted in middle age, with smaller dorsal field EPSPs detected in 10-14 month old mice compared to 2-3 month old mice (Schreurs, Sabanov and Balschun, 2017) and neurogenesis markers decline when rodents age from 4 months to 12 months in all hippocampal subregions (Hattiangady *et al.*, 2005). However, there appears to be subminimal investigation into whether hippocampal function is affected in earlier aging, and there appears to be no defined age at which hippocampal function and cognitive decline begins.

I therefore investigated whether activity along the perforant pathway, the main excitatory input into the hippocampus, was affected in early aging by comparing dentate gyrus excitability between juvenile (9-15 weeks old) and adult (25-35 weeks old) wild-type mice. My work is the first to report that population excitability in the dentate gyrus increased in early aging, which appeared to not be caused by post-synaptic activity, and instead could be a result of changes in pre-synaptic transmission. No age-related excitability changes were detected in the CA3 suggesting that the hippocampus as a whole structure does not undergo a widespread loss of connectional integrity during early aging and instead, perforant pathway connections from the entorhinal cortex layer II onto dentate granule neurons are specifically sensitive to early aging. Additionally, I observed that intrinsic membrane properties of granule neurons change during early aging, specifically in the supra-pyramidal region, which I hypothesise could be caused by reduction of sodium permeability, persistent sodium current or increased extracellular chloride concentrations. These findings contrast to experiments investigating dentate

gyrus excitability in late aging, which report a significant reduction in population excitability in the dentate gyrus during perforant pathway stimulation (Barnes, 1979; Barnes and McNaughton, 1980; Froc *et al.*, 2003). This suggests that dentate gyrus excitability may increase as rodents move into adulthood, but then decrease as the rodent transitions to an older age in a bell shaped curve. Dentate gyrus inhibitory currents follow a similar “bell shaped curve” during aging (Fleming, Wilson and Swartzwelder, 2007), suggesting that changes in population excitability in early aging could be caused by inhibitory pyramidal neurons influencing granule cell activity.

I also show that the supra-pyramidal dentate gyrus displays more prominent changes in early aging than the infra-pyramidal subregion. This data is novel and has relevance for previously reported functional (Fyhn *et al.*, 2004; Chawla *et al.*, 2005; Satvat *et al.*, 2011) and structural (Desmond and Levy, 1982; Desmond and Levy, 1985; Claiborne, Amaral and Cowan, 1990) differences between the dentate gyrus subregions. It is possible that excitatory transmission through the hippocampal tri-synaptic loop is routed more prominently through the supra-pyramidal dentate gyrus, as this subregion shows greater neuronal activity (Chawla *et al.*, 2013) due to a greater number of perforant path excitatory synapses (Sorra and Harris, 2000) than the infra-pyramidal subregion. If excitatory neurotransmission is routed predominantly through supra-pyramidal granule neurons to the CA3, it could explain why this subregion is specifically affected during aging and is directly associated with impaired memory performance in aging (Karaca *et al.*, 2021). This also highlights that the infra-pyramidal dentate gyrus subregion is more resilient to age-related alterations, potentially acting to regulate granule neuron response to aging and preventing a large increase of excitatory synaptic transmission across the whole subregion (Snyder *et al.*, 2009).

Neurophysiology experiments from animal models have highlighted that activity and function of the hippocampal tri-synaptic circuit is reduced in normal aging (Geinisman *et al.*, 1992; Bondareff and Geinisman, 1976; Barnes, 1979; Froc *et al.*, 2003) and this is directly associated with reduced cognition (Gage *et al.*, 1984; Mizumori, Lavoie and Kalyani, 1996; Shen *et al.*, 1997; Rapp, Kansky and Roberts, 1997). However, each subregion and its connections within the network contributes to a distinct set of properties underpinning memory function (Witter *et al.*, 2000), some of which are more sensitive to aging. Rodent models have highlighted that the perforant pathway is particularly

sensitive to age-related decline (Froc *et al.*, 2003; Barnes, Rao and Houston, 2000), which has been observed through a variety of immunofluorescent staining experiments (Bizon, Lee and Gallagher, 2004; Wang *et al.*, 2007; Fu *et al.*, 2015), *in vitro* electrophysiology measurements (Barnes, 1979; Barnes and McNaughton, 1980; Froc *et al.*, 2003), and *in vivo* recordings during hippocampal-dependent memory tests (Shen *et al.*, 1997; Bieri, Bobbitt and Colgin, 2014; Zheng *et al.*, 2016). Extrapolating this data in human models is much more challenging, and therefore the effects of aging on distinct hippocampal regions in humans is less well reported. Diffusion tensor imaging showed that the perforant-pathway in particular undergoes degeneration during normal aging which correlated with reduced memory performance (Yassa, Muftuler and Stark, 2010). Like most models of aging, these experiments compared perforant pathway activity between young (21 years old) and elderly (70 years old) human cohorts, and there is no longitudinal analysis exploring perforant pathway activity during progressive aging (Yassa, Muftuler and Stark, 2010). Longitudinal studies measuring the volume of brain sub-structures using magnetic resonance imaging (MRI) in human participants is commonly used as a prediction of clinical status (Grundman *et al.*, 2002). Reduced volume is associated with worsened clinical status and has been used to examine whether specific hippocampal subregions are affected during mild cognitive impairment or patients with neurodegenerative disease such as Alzheimer's disease (Scheff *et al.*, 2006; Wu *et al.*, 2008). Overall, the dentate gyrus and CA3 volume is more prominently reduced in normal aging (Wu *et al.*, 2008; Mueller and Weiner, 2009; Feng *et al.*, 2020). The dentate gyrus and CA3 are both implicated in age-related volume loss as they are often classified into one experimental group, due to the difficultly in distinguishing between the two subregions due to their proximity and MRI resolution. More recently, a longitudinal study using a high resolution MRI highlighted that human dentate gyrus volume decreases with increasing age whereas CA3 volume is age dependent (Dillon *et al.*, 2017). The dentate gyrus is important during pattern separation, whilst the CA3 is linked to the cognitive operation called pattern completion (Kent *et al.*, 2007; Lee *et al.*, 2004; Leutgeb *et al.*, 2004). Studies confirm that aging manifests with pattern separation deficits (Toner *et al.*, 2009; Yassa *et al.*, 2011), however performance on cognitive tasks that are sensitive to pattern completion are relatively preserved in aging (Toner *et al.*, 2009), supporting the idea that compared with the dentate gyrus, CA3 is relatively preserved (Dillon *et al.*, 2017).

During pathological aging differences in rate and extent of pathological events have been noted for different brain regions. The entorhinal cortex is the region most greatly affected in Alzheimer's disease, shown by pattern of cell death and volume loss (Van Hoesen, Hyman and Damasio, 1991; Braak *et al.*, 2006; GomezIsla *et al.*, 1996; Khan *et al.*, 2014), whereas the dentate gyrus and CA3 is mostly preserved (Mueller *et al.*, 2010). Furthermore, the reduction in synapse number in the outer molecular layer of the dentate gyrus in human brains with Alzheimer's disease compared to healthy individuals suggest that loss of afferents from the entorhinal cortex underlie synapse loss in early Alzheimer's disease (Scheff *et al.*, 2006). Because the entorhinal cortex is upstream to the dentate gyrus, cell death in this subregion is also expected to cause pattern separation deficits and defects in cognitive operations that are linked to other downstream subregions (Yassa *et al.*, 2011). The observed anatomical separation, with Alzheimer's disease differentially targeting the entorhinal cortex and aging targeting the dentate gyrus with relative sparing of the entorhinal cortex, suggests that Alzheimer's disease and aging have distinct pathologies.

6.3 CCL2 directly affects hippocampal function, but not in an age-dependent manner

The innate immune system is a crucial first line of defence immune response (Beutler, 2004) and prolonged periods of excessive inflammation can lead to tissue damage, especially in organs and tissues that are particularly sensitive to immune-mediated change such as the brain (Glezeva and Baugh, 2014; Ekdahl *et al.*, 2003; Vezzani and Granata, 2005). Although the brain has its own distinct immune system, immune components in the circulatory system such as chemokines and cytokines, cross the blood brain barrier and exert their influences in the cerebral environment (Gutierrez, Banks and Kastin, 1993).

The innate immune system becomes dysregulated with aging (Mahbub, Brubaker and Kovacs, 2011; Kovacs *et al.*, 2009; Chelvarajan *et al.*, 2006), and the inability to balance pro- and anti-inflammatory responses generates a basal elevation in pro-inflammatory mediators (Gomez *et al.*, 2007; Nomellini, Gomez and Kovacs, 2008). This general increase in inflammation is associated with changes in the regulation of neuronal processes underlying learning and memory including LTP and neurogenesis (Min *et al.*, 2009; Di Filippo *et al.*, 2013; Kohman and Rhodes, 2013), which can lead to

neurodegeneration and contribute directly to aging pathologies (Chung *et al.*, 2009; Glass *et al.*, 2010; Perry, Cunningham and Holmes, 2007) and cognitive decline (Weaver *et al.*, 2002; Wersching *et al.*, 2010; Bettcher *et al.*, 2012; Singh-Manoux *et al.*, 2014). These studies predominantly focus on inflammation during late aging, with human participants ranging between 63-79 years old and mice models 18-20 months old. A few studies have indicated that inflammation in the circulatory system and brain is upregulated in mid-aged individuals. Microglia from middle aged rodents (9 – 10 months old) have greater expression of proinflammatory cytokines than young rodents (2 months old) (Nikodemova *et al.*, 2016), and greater microglia proliferation in middle aged humans (30 – 54 years old) was associated with poorer spatial reasoning, learning and memory and executive function tests (Marsland *et al.*, 2015).

It is currently unclear how different proinflammatory chemokines impact hippocampal function, whether they act directly on neurons or indirectly via astroglia, and what the consequences are for neural activity. I investigated whether the proinflammatory chemokine CCL2 was responsible for increasing hippocampal excitability in early aging. CCL2 was an appropriate candidate for investigation as it is upregulated in the circulatory system during early aging (between mice of 3, 5, 12, 18 and +22 months old), is able to cross the blood brain barrier and cause proliferation of microglia, and also has been shown to directly affect neuronal activity in the CA1 and CA3 (Zhou *et al.*, 2011; Duan *et al.*, 2018; Yousefzadeh *et al.*, 2018). I report here that CCL2 directly increased perforant pathway excitability through its cognate receptor CCR2. This response was specific to CCL2, as the proinflammatory chemokine CCL5 did not significantly affect hippocampal excitability. However, there were no age-dependent effects of CCL2 on hippocampal excitability, suggesting that CCL2 is not regulating hippocampal excitability during early aging. Prolonged inflammation is associated with chemokine receptor desensitisation (Darbonne *et al.*, 1991), and therefore it is possible that increased circulatory levels of CCL2 during early aging could coincide with CCR2 desensitisation, which could also explain why less efficient immune responses are observed in aging (Weyand and Goronzy, 2016; Montecino-Rodriguez, Berent-Maoz and Dorshkind, 2013). These experiments suggests that either another pro-inflammatory molecule is regulating neuronal activity in early aging, or more likely, a combination of pro-inflammatory mediators upregulated in early aging is affecting hippocampal activity.

6.4 DARC regulates hippocampal excitability in early aging

The atypical non-signalling Duffy antigen receptor for chemokines (DARC) acts to regulate the concentration of proinflammatory chemokines in the blood, either by removing excess from circulation which dampens leukocyte activation (Dawson *et al.*, 2000; Zarbock *et al.*, 2010), or alternatively buffering chemokines and releasing them into circulation during an inflammatory response (Novitzky-Basso and Rot, 2012). DARC is expressed on red blood cells and the postcapillary endothelium (Segerer *et al.*, 2000) in a number of peripheral organs (Lee *et al.*, 2003; Kashiwazaki *et al.*, 2003; Hadley and Peiper, 1997) and regulates chemokine levels in the peripheral system. DARC expression has also been detected in neurons using immunohistochemical staining (Horuk *et al.*, 1997) and RNA-sequencing isolated by FACs (Schaum *et al.*, 2018), however its function in neurons is unknown. DARCs ability to regulate pro-inflammatory chemokine concentrations and its neuronal expression raised questions as to whether DARC played a role in regulating hippocampal excitability. I report here novel findings that DARC regulates early-age-dependent dentate gyrus excitability. I also report that the assumed DARC expression in dentate gyrus granule neurons regulates intrinsic membrane properties including resting membrane potential and membrane resistance, which change when aging from juvenile to adulthood. How DARC affects neuronal excitability without initiating intracellular signalling events is unknown. Despite this, DARC has previously been reported to affect the activity of other chemokine receptors through receptor dimerization, specifically to the chemokine receptor CCR5, where it impedes CCR5 receptor function by inhibiting calcium flux. The specific kinetics of this interaction is unknown; however, it is hypothesised that DARC inhibits receptor activity by altering G-protein activity (Chakera *et al.*, 2008). G-protein coupled receptors (GPCRs) play essential roles in neuronal communication, modulating pre- and post-synaptic activity (Moller *et al.*, 2018), and in particular the metabotropic glutamate receptor (mGlu) is a GPCR for the major excitatory neurotransmitter glutamate (Nicoletti *et al.*, 2015). These data suggest that DARC could be mediating hippocampal excitability through the disruption of hippocampal GPCRs such as mGLU and subsequently impeding excitatory transmission. I also observe that DARC regulates microglia proliferation in the dentate gyrus in early aging, and as microglia proliferation increases from young to mid age (3-4 months old to 13-14 months old) in the hippocampus (Mouton

et al., 2002), it suggests that DARC acts to mediate age-related hippocampal pathologies.

I identified no hippocampal-dependent behavioural changes between DARC knockout mice and wild-type mice, however other studies examining hippocampal-dependent behaviour using different behavioural models have indicated that DARC knockout mice have impaired spatial-navigation memory (Schneider *et al.*, 2014). These results suggest that DARC not only regulates hippocampal activity and microglia proliferating but is also required for cognitive function. Furthermore, my results and results in the literature collectively suggest that DARC knockout mice exhibit premature aging.

6.5 Limitations

In this thesis I have used electrophysiology, immunohistochemistry, and behavioural paradigms to report several novel aspects of hippocampal physiology. Here I discuss some of the limitations of my experimental approaches, which should be taken into account when interpreting and discussing the wider implications of my findings.

6.5.1 *In vitro* electrophysiology

Firstly, *in vitro* electrophysiology is used to throughout this thesis to investigate neuronal excitability, intrinsic cell properties and hippocampal oscillations. This is a useful technique which is a critical tool for assessing electrophysiological effects of pharmacological and genetic manipulations in the brain. Brain slices are kept in optimal conditions which allow for the recording of electrical properties of neurons, however this environment is artificial and does not accurately represent the physiological conditions of the body *in vivo*. Brain slices *in vitro* have no active circulatory system, therefore physiological responses which usually involve circulatory system influence, such as inflammatory processes which is investigated in this thesis, will not fully represent responses that would occur *in vivo*.

In the literature, there are sometimes contradictory outcomes to experiments performed using the same *in vitro* electrophysiology protocols. Although variations in equipment and technical set up can affect result outcomes, it is also important to note that conditions of the mice before slice preparation can affect biological measurements. For example, mice in high stress conditions before slice preparation can affect neuronal activity readouts, particularly oscillation measurements (Iturra-Mena *et al.*, 2019), and the time

of day of hippocampal slices preparation can effect excitability measurements due to circadian rhythms (Sakhi *et al.*, 2014). Lighting conditions, food availability and social interactions can also influence experimental outcomes (Dibner, Schibler and Albrecht, 2010; Piggins and Guilding, 2011; Bechtold and Loudon, 2013). For the experiments performed in this thesis I kept the slice preparation procedure as consistent as possible, with special consideration to reduce stress to mice before slice preparation, reducing handling and conducting the slice preparation at the same time each day.

6.5.2 Mice models to understand aging in humans

Laboratory mice have become a robust and reliable research tool in the field of aging due to their short life span, the ability of mouse tissue to be analysed at all stages of the aging process and the ability to create genetic mouse lines (Hasty and Vijg, 2004; Nadon, 2004). At the cellular level there are many similarities between the mouse and the human nervous system, however the mouse brain is less complex than the human brain (Vanhooren and Libert, 2013), and aging mice do not demonstrate all typical age-related diseases seen in humans. For example, cardiovascular diseases are the major causes of illness, disability and human mortality which are not reported in mouse models (Roger, 2011), and although mice show decreases in memory and cognition that parallel changes observed in the human population, they do not develop Alzheimer's disease (Jucker *et al.*, 1994). The study of aging in humans is a complex process, and interactions between biological changes, environmental and social factors, diet, genetic constitution and quality of healthcare throughout life are all factors which can affect vulnerability to age-related disease (Vanhooren and Libert, 2013). Meanwhile laboratory mice often have controlled diet, environment, and genetic background, although this does allow for the examination of senesce that exclude complex environmental factors.

Another caveat of using mice to model aging, is that there are rodent aging classification inconsistencies in the literature which gives rise to inconsistencies in some physiological reports of aging. Mice between 3-6 months of age are typically classified as young adults whilst animals aged 14-24+ months old are often pooled together in the single category of aged (Radulescu *et al.*, 2021). However more recent literature has investigated aging pathologies in middle age, which is generally categorised as mice that are 10-14 months old (Radulescu *et al.*, 2021; Schreurs, Sabanov and Balschun, 2017; Hattiangady *et al.*, 2005; Francia *et al.*, 2006), which overlaps with previous classifications of aged mice.

Physiological differences have been identified longitudinally with aging (Fleming, Wilson and Swartzwelder, 2007; Shoji *et al.*, 2016), which highlights that a broadly agreed age-classification of mice would help reduce variations in aging classification and terminology. Alternatively, clearly stating the ages of mice in the literature manuscript would also aid with transparency of the aging models used.

6.5.3 Acute CCL2 treatment as a model to understand effects of inflammation

When examining the effects of CCL2 on hippocampal excitability, I administered CCL2 at a concentration that is approximately 5 fold higher than concentrations measured in the brain after acute injury (Belkouch *et al.*, 2011). Although I used a similar CCL2 concentration as other electrophysiology studies measuring CCL2-mediated neuronal excitability (Gruol *et al.*, 2014; Guyon *et al.*, 2009; Zhou *et al.*, 2011; Belkouch *et al.*, 2011), it is possible that this higher amount of CCL2 is eliciting greater hippocampal activity than would occur during normal responses to inflammation.

During natural immune responses, a variety of chemokines and cytokines are upregulated in both the peripheral and central nervous system, which could all affect hippocampal function (Thomson *et al.*, 2020; Gyoneva and Ransohoff, 2015). Bath application of CCL2 onto hippocampal slices may not initiate neuronal responses that accurately reflect their activity during a more natural response to inflammation. Therefore, to measure hippocampal activity in response to inflammation that more accurately reflects the natural conditions in the brain, systemic inflammation could be induced in mice before dissecting the brain and measuring neuronal activity using *in vitro* electrophysiology. Systemic inflammation could be induced in mice using lipopolysaccharide injection, a common method of inducing and examining neuroinflammation (Batista *et al.*, 2019).

6.6 Concluding remarks and future work

I show for the first time that hippocampal excitability along the perforant pathway is increased in early aging and is associated with changes in intrinsic properties of granule neurons. As dentate gyrus excitability has been previously shown to decrease from middle to old age, my data suggests that hippocampal excitability does not follow a linear trajectory during aging, and instead may increase as rodents move into adulthood, but then decrease as the rodent transitions to an older age. I also identified that the increase in hippocampal activity during early aging is regulated by the atypical chemokine receptor DARC, its first identified function in the brain. This regulation involves changes in post-synaptic activity and intrinsic membrane properties of granule cell neurons of the dentate gyrus. I also show that DARC acts to regulate microglial proliferation in early aging, and CCL2-dependent microglia activation, highlighting that the receptor is involved in mediating several brain parameters in an age dependent manner.

The extent of DARCs role in regulating neuronal activity is unknown, and there are many more questions that need to be addressed. Most importantly, we need to develop a better understanding of where DARC is expressed in the brain, and on what neuronal populations it is expressed. This could be conducted using a chemokine receptor mapping kit, such as the Milliplex MAP mouse Cytokine/Chemokine Magnetic Bead Panel Kit (Covaris, USA). Multiple receptors and chemokines can be detected simultaneously and would not only highlight DARC concentration and its localisation in the brain, but also measure the expression of other chemokine receptors and pro-inflammatory chemokines. This would develop a better understanding of how DARC modulates chemokine concentrations in the brain and provide information about its localisation with other chemokine receptors. Furthermore, as DARC has functional roles in the circulatory system and on endothelial cells, it is important to detect whether DARC expression changes in these systems during aging, and how this expression relates to expression in the brain.

Additionally, how pro-inflammatory chemokines and their receptors affect hippocampal function is currently limited. The innate immune system is comprised of a complex network of pro-inflammatory, anti-inflammatory, and regulatory components that all may have individual and compound effects on various elements of brain function. Assessing how each component of inflammatory responses may affect brain activity is a large task,

however recognising these mechanisms may help to uncover how “inflammaging” leads to cognitive decline and the onset of neurodegenerative disorders, and lead to possible therapeutic interventions. Measuring all the chemokines that are upregulated in the hippocampus during systemic inflammation, induced by lipopolysaccharide injection for example, could be a good starting point. Once upregulated chemokines are identified, systematic investigation into how each chemokine individually effects hippocampal activity could help to identify a more complete picture of chemokine regulation in the region. Repeating these experiments in different rodent age cohorts could help to investigate whether sensitivity to these chemokines is affected during aging.

Appendix

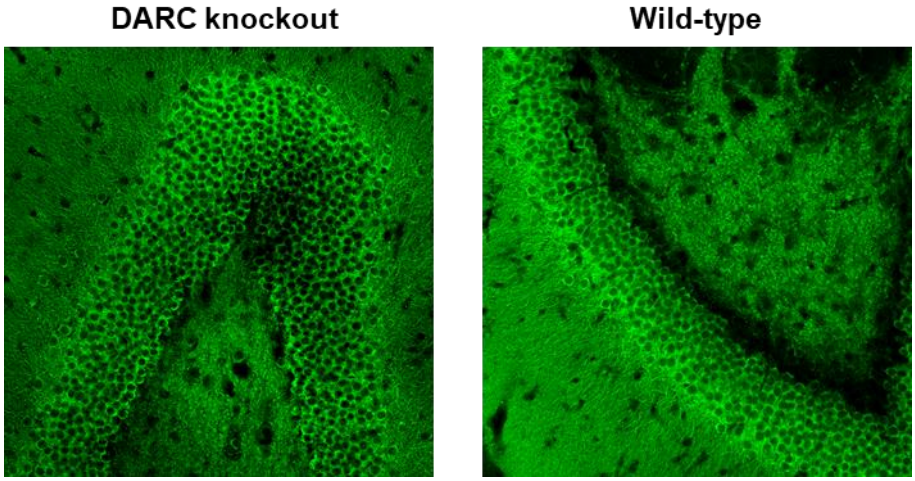


Figure 1 - Immunohistochemical staining of mouse hippocampal slices using an anti-DARC antibody showed positive staining in wild-type and DARC knockout mice. Hippocampal slices from wild-type and DARC knockout mice were collected, fixed in 4% PFA, cryoprotected with 30% sucrose in PBS, frozen in OCT and re-sectioned to 30µM slices. Free-floating immunofluorescence immunohistochemistry was conducted on the re-sectioned hippocampal slices. A rabbit monoclonal anti-DARC primary antibody (1:100, cat# ab137044, abcam) was used. DARC positive staining was observed in dentate gyrus neurons in wild-type and DARC knockout mice.

Bibliography

Aghajanian, G. K. and Rasmussen, K. (1989) 'Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices', *Synapse,* 3(4), pp. 331-8.

Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M. and Rossi, F. M. (2011) 'Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool', *Nat Neurosci,* 14(9), pp. 1142-9.

Akdogan, M. F., Azak, A., Denizli, N., Huddam, B., Kocak, G., Gucun, M., Tatlisu, M. A., Demirci, R., Yilmaz, B., Dikec, M., Bakirtas, M., Akdag, I. and Duranay, M. (2015) 'MCP-1 and soluble TWEAK levels are independently associated with coronary artery disease severity in patients with chronic kidney disease', *Renal Failure,* 37(8), pp. 1297-1302.

Allman, D. and Miller, J. P. (2005) 'B cell development and receptor diversity during aging', *Current Opinion in Immunology,* 17(5), pp. 463-467.

Alonso, A. and García-Austt, E. (1987) 'Neuronal sources of theta rhythm in the entorhinal cortex of the rat. II. Phase relations between unit discharges and theta field potentials', *Exp Brain Res,* 67(3), pp. 502-9.

Amaral, D. G. and Kurz, J. (1985) 'An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat', *J Comp Neurol,* 240(1), pp. 37-59.

Amaral, D. G., Scharfman, H. E. and Lavenex, P. (2007) 'The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies)', *Dentate Gyrus: a Comphrehensive Guide to Structure, Function, and Clinical Implications,* 163, pp. 3-+.

Amaral, D. G. and Witter, M. P. (1989) 'The three-dimensional organization of the hippocampal formation: a review of anatomical data', *Neuroscience,* 31(3), pp. 571-91.

Amdur, R. L., Feldman, H. I., Gupta, J., Yang, W., Kanetsky, P., Shlipak, M., Rahman, M., Lash, J. P., Townsend, R. R., Ojo, A., Roy-Chaudhury, A., Go, A. S., Joffe, M., He, J., Balakrishnan, V. S., Kimmel, P. L., Kusek, J. W., Raj, D. S. and Investigators, C. S. (2016) 'Inflammation and Progression of CKD: The CRIC Study', *Clinical Journal of the American Society of Nephrology,* 11(9), pp. 1546-1556.

Amenta, F., Bronzetti, E., Sabbatini, M. and Vega, J. A. (1998) 'Astrocyte changes in aging cerebral cortex and hippocampus: a quantitative immunohistochemical study', *Microsc Res Tech,* 43(1), pp. 29-33.

Anand, K. S. and Dhikav, V. (2012) 'Hippocampus in health and disease: An overview', *Annals of Indian Academy of Neurology,* 15(4), pp. 239-246.

Anderson, M. I. and Jeffery, K. J. (2003) 'Heterogeneous modulation of place cell firing by changes in context', *Journal of Neuroscience,* 23(26), pp. 8827-8835.

Antonelli, A., Rotondi, M., Fallahi, P., Ferrari, S. M., Paolicchi, A., Romagnani, P., Serio, M. and Ferrannini, E. (2006) 'Increase of CXC chemokine CXCL10 and CC chemokine CCL2 serum levels in normal ageing', *Cytokine,* 34(1-2), pp. 32-8.

Arango Duque, G. and Descoteaux, A. (2014) 'Macrophage cytokines: involvement in immunity and infectious diseases', *Front Immunol,* 5, pp. 491.

Arisi, G. M. (2014) 'Nervous and immune systems signals and connections: Cytokines in hippocampus physiology and pathology', *Epilepsy & Behavior,* 38, pp. 43-47.

Ashley, N. T., Weil, Z. M. and Nelson, R. J. (2012) 'Inflammation: Mechanisms, Costs, and Natural Variation', *Annual Review of Ecology, Evolution, and Systematics, Vol 43,* 43, pp. 385-406.

Ataka, K., Asakawa, A., Nagaishi, K., Kaimoto, K., Sawada, A., Hayakawa, Y., Tatezawa, R., Inui, A. and Fujimiya, M. (2013) 'Bone marrow-derived microglia infiltrate into the paraventricular nucleus of chronic psychological stress-loaded mice', *PLoS One,* 8(11), pp. e81744.

Bailey, S. L., Carpentier, P. A., McMahon, E. J., Begolka, W. S. and Miller, S. D. (2006) 'Innate and adaptive immune responses of the central nervous system', *Critical Reviews in Immunology,* 26(2), pp. 149-188.

Bak, J., Pyeon, H. I., Seok, J. I. and Choi, Y. S. (2017) 'Effect of rotation preference on spontaneous alternation behavior on Y maze and introduction of a new analytical method, entropy of spontaneous alternation', *Behavioural Brain Research,* 320, pp. 219-224.

Banisadr, G., Gosselin, R. D., Mechighel, P., Kitabgi, P., Rosene, W. and Parsadaniantz, S. P. M. (2005) 'Highly regionalized neuronal expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) in rat brain: Evidence for its colocalization with neurotransmitters and neuropeptides', *Journal of Comparative Neurology,* 489(3), pp. 275-292.

Banisadr, G., Queraud-Lesaux, F., Boutterin, M. C., Pelaprat, D., Zalc, B., Rostene, W., Haour, F. and Parsadaniantz, S. M. (2002) 'Distribution, cellular localization and functional role of CCR2 chemokine receptors in adult rat brain', *Journal of Neurochemistry,* 81(2), pp. 257-269.

Banisadr, G., Rostène, W., Kitabgi, P. and Parsadaniantz, S. M. (2005c) 'Chemokines and brain functions', *Curr Drug Targets Inflamm Allergy,* 4(3), pp. 387-99.

Bannerman, D. M., Deacon, R. M., Offen, S., Friswell, J., Grubb, M. and Rawlins, J. N. (2002) 'Double dissociation of function within the hippocampus: spatial memory and hyponeophagia', *Behav Neurosci,* 116(5), pp. 884-901.

Bannerman, D. M., Grubb, M., Deacon, R. M., Yee, B. K., Feldon, J. and Rawlins, J. N. (2003) 'Ventral hippocampal lesions affect anxiety but not spatial learning', *Behav Brain Res,* 139(1-2), pp. 197-213.

Bannerman, D. M., Rawlins, J. N., McHugh, S. B., Deacon, R. M., Yee, B. K., Bast, T., Zhang, W. N., Pothuizen, H. H. and Feldon, J. (2004) 'Regional dissociations within the hippocampus--memory and anxiety', *Neurosci Biobehav Rev,* 28(3), pp. 273-83.

Bannerman, D. M., Sprengel, R., Sanderson, D. J., McHugh, S. B., Rawlins, J. N., Monyer, H. and Seeburg, P. H. (2014) 'Hippocampal synaptic plasticity, spatial memory and anxiety', *Nat Rev Neurosci: Vol. 3*. England, pp. 181-92.

Barnes, C. A. (1979) 'Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat', *J Comp Physiol Psychol,* 93(1), pp. 74-104.

Barnes, C. A. and McNaughton, B. L. (1980) 'Physiological compensation for loss of afferent synapses in rat hippocampal granule cells during senescence', *J Physiol,* 309, pp. 473-85.

Barnes, C. A. and McNaughton, B. L. (1985) 'An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses', *Behav Neurosci,* 99(6), pp. 1040-8.

Barnes, C. A., Rao, G. and Houston, F. P. (2000a) 'LTP induction threshold change in old rats at the perforant path--granule cell synapse', *Neurobiol Aging,* 21(5), pp. 613-20.

Barrientos, R. M., Higgins, E. A., Sprunger, D. B., Watkins, L. R., Rudy, J. W. and Maier, S. F. (2002) 'Memory for context is impaired by a post context exposure injection of interleukin-1 beta into dorsal hippocampus', *Behavioural Brain Research,* 134(1-2), pp. 291-298.

Bartsch, T., Dohring, J., Reuter, S., Finke, C., Rohr, A., Brauer, H., Deuschl, G. and Jansen, O. (2015) 'Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging', *Journal of Cerebral Blood Flow and Metabolism,* 35(11), pp. 1836-1845.

Bartsch, T. and Wulff, P. (2015) 'The hippocampus in aging and disease: From plasticity to vulnerability', *Neuroscience,* 309, pp. 1-16.

Basu, J. and Siegelbaum, S. A. (2015) 'The Corticohippocampal Circuit, Synaptic Plasticity, and Memory', *Cold Spring Harbor Perspectives in Biology,* 7(11), pp. 26.

Batista, C. R. A., Gomes, G. F., Candelario-Jalil, E., Fiebich, B. L. and de Oliveira, A. C. P. (2019) 'Lipopolysaccharide-Induced Neuroinflammation as a Bridge to Understand Neurodegeneration', *International Journal of Molecular Sciences,* 20(9).

Bazzigaluppi, P., Beckett, T. L., Koletar, M. M., Lai, A. Y., Joo, I. L., Brown, M. E., Carlen, P. L., McLaurin, J. and Stefanovic, B. (2018) 'Early-stage attenuation of phase-amplitude coupling in the hippocampus and medial prefrontal cortex in a transgenic rat model of Alzheimer's disease', *J Neurochem,* 144(5), pp. 669-679.

Bechtold, D. A. and Loudon, A. S. (2013) 'Hypothalamic clocks and rhythms in feeding behaviour', *Trends Neurosci,* 36(2), pp. 74-82.

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., Siklos, L., McKercher, S. R. and Appel, S. H. (2006) 'Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis', *Proc Natl Acad Sci U S A,* 103(43), pp. 16021-6.

Belkouch, M., Dansereau, M. A., Goazigo, A. R. L., Van Steenwinckel, J., Beaudet, N., Chraibi, A., Melik-Parsadaniantz, S. and Sarret, P. (2011) 'The Chemokine CCL2 Increases Na(v)1.8 Sodium Channel Activity in Primary Sensory Neurons through a G beta gamma-Dependent Mechanism', *Journal of Neuroscience,* 31(50), pp. 18381-18390.

Benarroch, E. E. (2013) 'HCN channels Function and clinical implications', *Neurology,* 80(3), pp. 304-310.

Benice, T. S., Rizk, A., Kohama, S., Pfankuch, T. and Raber, J. (2006) 'Sex-differences in age-related cognitive decline in C57BL/6J mice associated with increased brain microtubule-associated protein 2 and synaptophysin immunoreactivity', *Neuroscience,* 137(2), pp. 413-423.

Benson, T. W., Weintraub, D. S., Crowe, M., Yiew, N. K. H., Popoola, O., Pillai, A., Joseph, J., Archer, K., Greenway, C., Chatterjee, T. K., Mintz, J., Stepp, D. W., Stansfield, B. K., Chen, W., Brittain, J., Bogdanov, V. Y., Gao, Y., Wilson, J. G., Tang, Y., Kim, H. W. and Weintraub, N. L. (2018) 'Deletion of the Duffy antigen receptor for chemokines (DARC) promotes insulin resistance and adipose tissue inflammation during high fat feeding', *Mol Cell Endocrinol,* 473, pp. 79-88.

Berry, R. J. and Bronson, F. H. (1992) 'Life history and bioeconomy of the house mouse', *Biol Rev Camb Philos Soc,* 67(4), pp. 519-50.

Bettcher, B. M., Fitch, R., Wynn, M. J., Lalli, M. A., Elofson, J., Jastrzab, L., Mitic, L., Miller, Z. A., Rabinovici, G. D., Miller, B. L., Kao, A. W., Kosik, K. S. and Kramer, J. H. (2016) 'MCP-1 and eotaxin-1 selectively and negatively associate with memory in MCI

and Alzheimer's disease dementia phenotypes', *Alzheimers Dement (Amst),* 3, pp. 91-7.

Bettcher, B. M., Neuhaus, J., Wynn, M. J., Elahi, F. M., Casaletto, K. B., Saloner, R., Fitch, R., Karydas, A. and Kramer, J. H. (2019) 'Increases in a Pro-inflammatory Chemokine, MCP-1, Are Related to Decreases in Memory Over Time', *Frontiers in Aging Neuroscience,* 11.

Bettcher, B. M., Wilheim, R., Rigby, T., Green, R., Miller, J. W., Racine, C. A., Yaffe, K., Miller, B. L. and Kramer, J. H. (2012) 'C-reactive protein is related to memory and medial temporal brain volume in older adults', *Brain Behav Immun,* 26(1), pp. 103-8.

Bettio, L. E. B., Rajendran, L. and Gil-Mohapel, J. (2017) 'The effects of aging in the hippocampus and cognitive decline', *Neuroscience and Biobehavioral Reviews,* 79, pp. 66-86.

Beutler, B. (2004) 'Innate immunity: an overview', *Mol Immunol,* 40(12), pp. 845-59.

Bieri, K. W., Bobbitt, K. N. and Colgin, L. L. (2014) 'Slow and fast γ rhythms coordinate different spatial coding modes in hippocampal place cells', *Neuron,* 82(3), pp. 670-81.

Bird, C. M. and Burgess, N. (2008) 'The hippocampus and memory: insights from spatial processing', *Nature Reviews Neuroscience,* 9(3), pp. 182-194.

Bizon, J. L., Lee, H. J. and Gallagher, M. (2004) 'Neurogenesis in a rat model of age-related cognitive decline', *Aging Cell,* 3(4), pp. 227-234.

Blackstad, T. W., Brink, K., Hem, J. and Jeune, B. (1970) 'Distribution of hippocampal mossy fibers in the rat. An experimental study with silver impregnation methods', *J Comp Neurol,* 138(4), pp. 433-49.

Blatteis, C. M., Bealer, S. L., Hunter, W. S., Llanos, Q. J., Ahokas, R. A. and Mashburn, T. A., Jr. (1983) 'Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs', *Brain Res Bull,* 11(5), pp. 519-26.

Blau, C. W., Cowley, T. R., O'Sullivan, J., Grehan, B., Browne, T. C., Kelly, L., Birch, A., Murphy, N., Kelly, A. M., Kerskens, C. M. and Lynch, M. A. (2012) 'The age-related deficit in LTP is associated with changes in perfusion and blood-brain barrier permeability', *Neurobiology of Aging,* 33(5).

Bliss, T. and Schoepfer, R. (2004) 'Controlling the ups and downs of synaptic strength', *Science,* 304(5673), pp. 973-974.

Bluestone, J. A. and Abbas, A. K. (2003) 'Natural versus adaptive regulatory T cells', *Nature Reviews Immunology,* 3(3), pp. 253-257.

Bondareff, W. and Geinisman, Y. (1976) 'Loss of synapses in the dentate gyrus of the senescent rat', *Am J Anat,* 145(1), pp. 129-36.

Bone, R. C. (1991) 'Gram-negative sepsis. Background, clinical features, and intervention', *Chest,* 100(3), pp. 802-8.

Bonecchi, R. and Graham, G. J. (2016) 'Atypical Chemokine Receptors and Their Roles in the Resolution of the inflammatory Response', *Frontiers in Immunology,* 7.

Bonne, O., Vythilingam, M., Inagaki, M., Wood, S., Neumeister, A., Nugent, A. C., Snow, J., Luckenbaugh, D. A., Bain, E. E., Drevets, W. C. and Charney, D. S. (2008) 'Reduced posterior hippocampal volume in posttraumatic stress disorder', *Journal of Clinical Psychiatry,* 69(7), pp. 1087-1091.

Bontempi, B., Laurent-Demir, C., Destrade, C. and Jaffard, R. (1999) 'Time-dependent reorganization of brain circuitry underlying long-term memory storage', *Nature,* 400(6745), pp. 671-675.

Bowman, G. L., Dayon, L., Kirkland, R., Wojcik, J., Peyratout, G., Severin, I. C., Henry, H., Oikonomidi, A., Migliavacca, E., Bacher, M. and Popp, J. (2019) 'Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults (vol 14, pg 1640, 2018)', *Alzheimers & Dementia,* 15(2), pp. 319-319.

Boyle, P. A., Wilson, R. S., Yu, L., Barr, A. M., Honer, W. G., Schneider, J. A. and Bennett, D. A. (2013) 'Much of late life cognitive decline is not due to common neurodegenerative pathologies', *Ann Neurol,* 74(3), pp. 478-89.

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H. and Del Tredici, K. (2006) 'Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry', *Acta Neuropathologica,* 112(4), pp. 389-404.

Braak, H. and Braak, E. (1991) 'Neuropathological stageing of Alzheimer-related changes', *Acta Neuropathol,* 82(4), pp. 239-59.

Bragin, A., Jandó, G., Nádasdy, Z., Hetke, J., Wise, K. and Buzsáki, G. (1995) 'Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat', *J Neurosci,* 15(1 Pt 1), pp. 47-60.

Bramham, C. R., Worley, P. F., Moore, M. J. and Guzowski, J. F. (2008) 'The Immediate Early Gene Arc/Arg3.1: Regulation, Mechanisms, and Function', *Journal of Neuroscience,* 28(46), pp. 11760-11767.

Breder, C. D., Hazuka, C., Ghayur, T., Klug, C., Huginin, M., Yasuda, K., Teng, M. and Saper, C. B. (1994) 'Regional induction of tumor necrosis factor alpha expression in the mouse brain after systemic lipopolysaccharide administration', *Proc Natl Acad Sci U S A,* 91(24), pp. 11393-7.

Broadbent, N. J., Squire, L. R. and Clark, R. E. (2004) 'Spatial memory, recognition memory, and the hippocampus', *Proc Natl Acad Sci U S A,* 101(40), pp. 14515-20.

Brubaker, A. L., Palmer, J. L. and Kovacs, E. J. (2011) 'Age-related Dysregulation of Inflammation and Innate Immunity: Lessons Learned from Rodent Models', *Aging and Disease,* 2(5), pp. 346-360.

Bruunsgaard, H., Andersen-Ranberg, K., Hjelmborg, J., Pedersen, B. K. and Jeune, B. (2003) 'Elevated levels of tumor necrosis factor alpha and mortality in centenarians', *Am J Med,* 115(4), pp. 278-83.

Bruunsgaard, H., Andersen-Ranberg, K., Jeune, B., Pedersen, A. N., Skinhøj, P. and Pedersen, B. K. (1999) 'A high plasma concentration of TNF-alpha is associated with dementia in centenarians', *J Gerontol A Biol Sci Med Sci,* 54(7), pp. M357-64.

Buchanan, T. W., Tranel, D. and Kirschbaum, C. (2009) 'Hippocampal damage abolishes the cortisol response to psychosocial stress in humans', *Hormones and Behavior,* 56(1), pp. 44-50.

Buhl, E. H., Han, Z. S., Lörinczi, Z., Stezhka, V. V., Karnup, S. V. and Somogyi, P. (1994) 'Physiological properties of anatomically identified axo-axonic cells in the rat hippocampus', *J Neurophysiol,* 71(4), pp. 1289-307.

Buhl, E. H., Szilágyi, T., Halasy, K. and Somogyi, P. (1996) 'Physiological properties of anatomically identified basket and bistratified cells in the CA1 area of the rat hippocampus in vitro', *Hippocampus,* 6(3), pp. 294-305.

Burger, C. (2010) 'Region-specific genetic alterations in the aging hippocampus: implications for cognitive aging', *Frontiers in Aging Neuroscience,* 2, pp. 12.

Burke, S. N. and Barnes, C. A. (2010) 'Senescent synapses and hippocampal circuit dynamics', *Trends in Neurosciences,* 33(3), pp. 153-161.

Burwell, R. D. (2000) 'The parahippocampal region: corticocortical connectivity', *Ann N Y Acad Sci,* 911, pp. 25-42.

Burwell, R. D. and Amaral, D. G. (1998) 'Perirhinal and postrhinal cortices of the rat: Interconnectivity and connections with the entorhinal cortex', *Journal of Comparative Neurology,* 391(3), pp. 293-321.

Buzsaki, G. (2005) 'Theta rhythm of navigation: Link between path integration and landmark navigation, episodic and semantic memory', *Hippocampus,* 15(7), pp. 827-840.

Buzsaki, G. and Moser, E. I. (2013) 'Memory, navigation and theta rhythm in the hippocampal-entorhinal system', *Nature Neuroscience,* 16(2), pp. 130-138.

Buzsáki, G. and Wang, X. J. (2012) 'Mechanisms of gamma oscillations', *Annu Rev Neurosci,* 35, pp. 203-25.

Byrne, A. and Reen, D. J. (2002) 'Lipopolysaccharide induces rapid production of IL-10 by monocytes in the presence of apoptotic neutrophils', *J Immunol,* 168(4), pp. 1968-77.

Calder, A. J., Lawrence, A. D. and Young, A. W. (2001) 'Neuropsychology of fear and loathing', *Nat Rev Neurosci,* 2(5), pp. 352-63.

Camicioli, R., Moore, M. M., Kinney, A., Corbridge, E., Glassberg, K. and Kaye, J. A. (2003) 'Parkinson's disease is associated with hippocampal atrophy', *Mov Disord,* 18(7), pp. 784-90.

Campuzano, O., Castillo-Ruiz, M. M., Acarin, L., Castellano, B. and Gonzalez, B. (2009) 'Increased Levels of Proinflammatory Cytokines in the Aged Rat Brain Attenuate Injury-induced Cytokine Response After Excitotoxic Damage', *Journal of Neuroscience Research,* 87(11), pp. 2484-2498.

Canto, C. B., Wouterlood, F. G. and Witter, M. P. (2008) 'What does the anatomical organization of the entorhinal cortex tell us?', *Neural Plast,* 2008, pp. 381243.

Castellano, J. M., Mosher, K. I., Abbey, R. J., McBride, A. A., James, M. L., Berdnik, D., Shen, J. C., Zou, B. D., Xie, X. M. S., Tingle, M., Hinkson, I. V., Angst, M. S. and Wyss-Coray, T. (2017) 'Human umbilical cord plasma proteins revitalize hippocampal function in aged mice', *Nature,* 544(7651), pp. 488-+.

Cerbai, F., Lana, D., Nosi, D., Petkova-Kirova, P., Zecchi, S., Brothers, H. M., Wenk, G. L. and Giovannini, M. G. (2012) 'The neuron-astrocyte-microglia triad in normal brain ageing and in a model of neuroinflammation in the rat hippocampus', *PLoS One,* 7(9), pp. e45250.

Chakera, A., Seeber, R. M., John, A. E., Eidne, K. A. and Greaves, D. R. (2008) 'The Duffy antigen/receptor for chemokines exists in an oligomeric form in living cells and functionally antagonizes CCR5 signaling through hetero-oligomerization', *Molecular Pharmacology,* 73(5), pp. 1362-1370.

Chambers, D. A., Cohen, R. L. and Perlman, R. L. (1993) 'Neuroimmune modulation: signal transduction and catecholamines', *Neurochem Int,* 22(2), pp. 95-110.

Chapman, C. A. and Lacaille, J. C. (1999) 'Cholinergic induction of theta-frequency oscillations in hippocampal inhibitory interneurons and pacing of pyramidal cell firing', *J Neurosci,* 19(19), pp. 8637-45.

Charo, I. F. and Ransohoff, R. M. (2006) 'Mechanisms of disease - The many roles of chemokines and chemokine receptors in inflammation', *New England Journal of Medicine,* 354(6), pp. 610-621.

Chawla, M. K., Guzowski, J. F., Ramirez-Amaya, V., Lipa, P., Hoffman, K. L., Marriott, L. K., Worley, P. F., McNaughton, B. L. and Barnes, C. A. (2005) 'Sparse, environmentally selective expression of arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience', *Hippocampus,* 15(5), pp. 579-586.

Chawla, M. K., Penner, M. R., Olson, K. M., Sutherland, V. L., Mittelman-Smith, M. A. and Barnes, C. A. (2013) 'Spatial behavior and seizure-induced changes in c-fos mRNA expression in young and old rats', *Neurobiology of Aging,* 34(4), pp. 1184-1198.

Chelvarajan, R. L., Liu, Y. S., Popa, D., Getchell, M. L., Getchell, T. V., Stromberg, A. J. and Bondada, S. (2006) 'Molecular basis of age-associated cytokine dysregulation in LPS-stimulated macrophages', *Journal of Leukocyte Biology,* 79(6), pp. 1314-1327.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X. and Zhao, L. (2018) 'Inflammatory responses and inflammation-associated diseases in organs', *Oncotarget,* 9(6), pp. 7204-7218.

Chen, W. W., Zhang, X. and Huang, W. J. (2016) 'Role of neuroinflammation in neurodegenerative diseases', *Molecular Medicine Reports,* 13(4), pp. 3391-3396.

Cherry, J. D., Meng, G. Y., Daley, S., Xia, W. M., Svirsky, S., Alvarez, V. E., Nicks, R., Pothast, M., Kelley, H., Huber, B., Tripodis, Y., Alosco, M. L., Mez, J., McKee, A. C. and Stein, T. D. (2020) 'CCL2 is associated with microglia and macrophage recruitment in chronic traumatic encephalopathy', *Journal of Neuroinflammation,* 17(1).

Chiao, Y. A., Dai, Q. X., Zhang, J. H., Lin, J., Lopez, E. F., Ahuja, S. S., Chou, Y. M., Lindsey, M. L. and Jin, Y. F. (2011) 'Multi-Analyte Profiling Reveals Matrix Metalloproteinase-9 and Monocyte Chemotactic Protein-1 as Plasma Biomarkers of Cardiac Aging', *Circulation-Cardiovascular Genetics,* 4(4), pp. 455-462.

Choi, S. S., Lee, H. J., Lim, I., Satoh, J. and Kim, S. U. (2014) 'Human Astrocytes: Secretome Profiles of Cytokines and Chemokines', *Plos One,* 9(4).

Chung, H. Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A. Y., Carter, C., Yu, B. P. and Leeuwenburgh, C. (2009) 'Molecular inflammation: Underpinnings of aging and age-related diseases', *Ageing Research Reviews,* 8(1), pp. 18-30.

Claiborne, B. J., Amaral, D. G. and Cowan, W. M. (1990) 'Quantitative, three-dimensional analysis of granule cell dendrites in the rat dentate gyrus', *J Comp Neurol,* 302(2), pp. 206-19.

Clarke, L. E., Liddelow, S. A., Chakraborty, C., Münch, A. E., Heiman, M. and Barres, B. A. (2018) 'Normal aging induces A1-like astrocyte reactivity', *Proc Natl Acad Sci U S A,* 115(8), pp. E1896-e1905.

Clayton, N. S. and Dickinson, A. (1998) 'Episodic-like memory during cache recovery by scrub jays', *Nature,* 395(6699), pp. 272-274.

Clayton, N. S., Salwiczek, L. H. and Dickinson, A. (2007) 'Episodic memory', *Curr Biol,* 17(6), pp. R189-91.

Cohen, O., Reichenberg, A., Perry, C., Ginzberg, D., Pollmächer, T., Soreq, H. and Yirmiya, R. (2003) 'Endotoxin-induced changes in human working and declarative memory associate with cleavage of plasma "readthrough" acetylcholinesterase', *J Mol Neurosci,* 21(3), pp. 199-212.

Colgin, L. L., Denninger, T., Fyhn, M., Hafting, T., Bonnevie, T., Jensen, O., Moser, M. B. and Moser, E. I. (2009) 'Frequency of gamma oscillations routes flow of information in the hippocampus', *Nature,* 462(7271), pp. 353-U119.

Collatz, M. B., Rüdel, R. and Brinkmeier, H. (1997) 'Intracellular calcium chelator BAPTA protects cells against toxic calcium overload but also alters physiological calcium responses', *Cell Calcium,* 21(6), pp. 453-9.

Colom, L. V., Castaneda, M. T., Reyna, T., Hernandez, S. and Garrido-Sanabria, E. (2005) 'Characterization of medial septal glutamatergic neurons and their projection to the hippocampus', *Synapse,* 58(3), pp. 151-64.

Colonna, M. and Butovsky, O. (2017) 'Microglia Function in the Central Nervous System During Health and Neurodegeneration', *Annual Review of Immunology, Vol 35,* 35, pp. 441-468.

Conde, J. R. and Streit, W. J. (2006) 'Microglia in the aging brain', *J Neuropathol Exp Neurol,* 65(3), pp. 199-203.

Conway, M. A. (2009) 'Episodic memories', *Neuropsychologia,* 47(11), pp. 2305-13.

Coppe, J. P., Desprez, P. Y., Krtolica, A. and Campisi, J. (2010) 'The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression', *Annual Review of Pathology-Mechanisms of Disease,* 5, pp. 99-118.

Corbisier, J., Galès, C., Huszagh, A., Parmentier, M. and Springael, J. Y. (2015) 'Biased signaling at chemokine receptors', *J Biol Chem,* 290(15), pp. 9542-54.

Cortez, M. A., Li, C., Whitehead, S. N., Dhani, S. U., D'Antonio, C., Huan, L. J., Bennett, S. A., Snead, O. C., 3rd and Bear, C. E. (2010) 'Disruption of ClC-2 expression is associated with progressive neurodegeneration in aging mice', *Neuroscience,* 167(1), pp. 154-62.

Costall, B., Jones, B. J., Kelly, M. E., Naylor, R. J. and Tomkins, D. M. (1989) 'Exploration of mice in a black and white test box: validation as a model of anxiety', *Pharmacol Biochem Behav,* 32(3), pp. 777-85.

Cramer, S. C., Sur, M., Dobkin, B. H., O'Brien, C., Sanger, T. D., Trojanowski, J. Q., Rumsey, J. M., Hicks, R., Cameron, J., Chen, D. F., Chen, W. G., Cohen, L. G., deCharms, C., Duffy, C. J., Eden, G. F., Fetz, E. E., Filart, R., Freund, M., Grant, S. J., Haber, S., Kalivas, P. W., Kolb, B., Kramer, A. F., Lynch, M., Mayberg, H. S., McQuillen, P. S., Nitkin, R., Pascual-Leone, A., Reuter-Lorenz, P., Schiff, N., Sharma, A., Shekim, L., Stryker, M., Sullivan, E. V. and Vinogradov, S. (2011) 'Harnessing neuroplasticity for clinical applications', *Brain,* 134, pp. 1591-1609.

Critchley, H., Daly, E., Phillips, M., Brammer, M., Bullmore, E., Williams, S., Van Amelsvoort, T., Robertson, D., David, A. and Murphy, D. (2000) 'Explicit and implicit neural mechanisms for processing of social information from facial expressions: a functional magnetic resonance imaging study', *Hum Brain Mapp,* 9(2), pp. 93-105.

Crone, N. E., Miglioretti, D. L., Gordon, B. and Lesser, R. P. (1998) 'Functional mapping of human sensorimotor cortex with electrocorticographic spectral analysis. II. Event-related synchronization in the gamma band', *Brain,* 121 (Pt 12), pp. 2301-15.

Cuadros, M. A. and Navascués, J. (1998) 'The origin and differentiation of microglial cells during development', *Prog Neurobiol,* 56(2), pp. 173-89.

Cunningham, C., Campion, S., Lunnon, K., Murray, C. L., Woods, J. F., Deacon, R. M., Rawlins, J. N. and Perry, V. H. (2009) 'Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease', *Biol Psychiatry,* 65(4), pp. 304-12.

Cunningham, C. and Sanderson, D. J. (2008) 'Malaise in the water maze: Untangling the effects of LPS and IL-1 beta on learning and memory', *Brain Behavior and Immunity,* 22(8), pp. 1117-1127.

Cunningham, M. O., Davies, C. H., Buhl, E. H., Kopell, N. and Whittington, M. A. (2003) 'Gamma oscillations induced by kainate receptor activation in the entorhinal cortex in vitro', *Journal of Neuroscience,* 23(30), pp. 9761-9769.

Cunningham, M. O., Whittington, M. A., Bibbig, A., Roopun, A., LeBeau, F. E., Vogt, A., Monyer, H., Buhl, E. H. and Traub, R. D. (2004) 'A role for fast rhythmic bursting neurons in cortical gamma oscillations in vitro', *Proc Natl Acad Sci U S A,* 101(18), pp. 7152-7.

Cutsuridis, V., Cobb, S. and Graham, B. P. (2010) 'Encoding and Retrieval in a Model of the Hippocampal CA1 Microcircuit', *Hippocampus,* 20(3), pp. 423-446.

Czeh, B., Abraham, H., Tahtakran, S., Houser, C. R. and Seress, L. (2013) Number and regional distribution of GAD65 nMRNA-expressing interneurons in rat hippocampal formation'', *Acta Biologica Hungarica,* 64(4), pp. 395-413.

Damani, M. R., Zhao, L. A., Fontainhas, A. M., Amaral, J., Fariss, R. N. and Wong, W. T. (2011) 'Age-related alterations in the dynamic behavior of microglia', *Aging Cell,* 10(2), pp. 263-276.

Dantzer, R., Aubert, A., Bluthé, R. M., Gheusi, G., Cremona, S., Layé, S., Konsman, J. P., Parnet, P. and Kelley, K. W. (1999) 'Mechanisms of the behavioural effects of cytokines', *Adv Exp Med Biol,* 461, pp. 83-105.

Dantzer, R., Konsman, J. P., Bluthé, R. M. and Kelley, K. W. (2000) 'Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent?', *Auton Neurosci,* 85(1-3), pp. 60-5.

Darbonne, W. C., Rice, G. C., Mohler, M. A., Apple, T., Hébert, C. A., Valente, A. J. and Baker, J. B. (1991) 'Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin', *J Clin Invest,* 88(4), pp. 1362-9.

Davidson, P. S., Drouin, H., Kwan, D., Moscovitch, M. and Rosenbaum, R. S. (2012) 'Memory as social glue: close interpersonal relationships in amnesic patients', *Front Psychol,* 3, pp. 531.

Davis, E. J., Foster, T. D. and Thomas, W. E. (1994) 'Cellular forms and functions of brain microglia', *Brain Res Bull,* 34(1), pp. 73-8.

Davoust, N., Vuaillat, C., Androdias, G. and Nataf, S. (2008) 'From bone marrow to microglia: barriers and avenues', *Trends Immunol,* 29(5), pp. 227-34.

Dawson, T. C., Lentsch, A. B., Wang, Z. X., Cowhig, J. E., Rot, A., Maeda, N. and Peiper, S. C. (2000) 'Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC)', *Blood,* 96(5), pp. 1681-1684.

de Kloet, E. R., Joëls, M. and Holsboer, F. (2005) 'Stress and the brain: from adaptation to disease', *Nat Rev Neurosci,* 6(6), pp. 463-75.

de Kloet, E. R., Karst, H. and Joëls, M. (2008) 'Corticosteroid hormones in the central stress response: quick-and-slow', *Front Neuroendocrinol,* 29(2), pp. 268-72.

De la Fuente, M. and Miquel, J. (2009) 'An Update of the Oxidation-Inflammation Theory of Aging: The Involvement of the Immune System in Oxi-Inflamm-Aging', *Current Pharmaceutical Design,* 15(26), pp. 3003-3026.

de No, R. L. (1933) 'Studies on the structure of the cerebral cortex I The area entorhinalis', *Journal Fur Psychologie Und Neurologie,* 45, pp. 381-438.

de Paz, J. L., Moseman, E. A., Noti, C., Polito, L., von Andrian, U. H. and Seeberger, P. H. (2007) 'Profiling heparin-chemokine interactions using synthetic tools', *ACS Chem Biol,* 2(11), pp. 735-44.

Dedovic, K., Duchesne, A., Andrews, J., Engert, V. and Pruessner, J. C. (2009) 'The brain and the stress axis: The neural correlates of cortisol regulation in response to stress', *Neuroimage,* 47(3), pp. 864-871.

Del Castillo, J. and Katz, B. (1954) 'Quantal components of the end-plate potential', *J Physiol,* 124(3), pp. 560-73.

Denenberg, V. H. (1969) 'Open-field bheavior in the rat: what does it mean?', *Ann N Y Acad Sci,* 159(3), pp. 852-9.

Deo, R., Khera, A., McGuire, D. K., Murphy, S. A., Neto, J., Morrow, D. A. and de Lemos, J. A. (2004) 'Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis', *Journal of the American College of Cardiology,* 44(9), pp. 1812-1818.

Deshmane, S. L., Kremlev, S., Amini, S. and Sawaya, B. E. (2009) 'Monocyte Chemoattractant Protein-1 (MCP-1): An Overview', *Journal of Interferon and Cytokine Research,* 29(6), pp. 313-326.

Deshmukh, S. S. and Knierim, J. J. (2011) 'Representation of non-spatial and spatial information in the lateral entorhinal cortex', *Frontiers in Behavioral Neuroscience,* 5, pp. 33.

Desmond, N. L. and Levy, W. B. (1982) 'A quantitative anatomical study of the granule cell dendritic fields of the rat dentate gyrus using a novel probabilistic method', *J Comp Neurol,* 212(2), pp. 131-45.

Desmond, N. L. and Levy, W. B. (1985) 'Granule cell dendritic spine density in the rat hippocampus varies with spine shape and location', *Neuroscience Letters,* 54(2-3), pp. 219-224.

Devreotes, P. N. and Zigmond, S. H. (1988) 'Chemotaxis in eukaryotic cells: a focus on leukocytes and Dictyostelium', *Annu Rev Cell Biol,* 4, pp. 649-86.

Dhillon, N. K., Williams, R., Callen, S., Zien, C., Narayan, O. and Buch, S. (2008) 'Roles of MCP-1 in development of HIV-dementia', *Frontiers in Bioscience,* 13, pp. 3913-3918.

Di Filippo, M., Chiasserini, D., Gardoni, F., Viviani, B., Tozzi, A., Giampà, C., Costa, C., Tantucci, M., Zianni, E., Boraso, M., Siliquini, S., de Iure, A., Ghiglieri, V., Colcelli, E., Baker, D., Sarchielli, P., Fusco, F. R., Di Luca, M. and Calabresi, P. (2013) 'Effects of central and peripheral inflammation on hippocampal synaptic plasticity', *Neurobiol Dis,* 52, pp. 229-36.

Di Giorgio, F. P., Carrasco, M. A., Siao, M. C., Maniatis, T. and Eggan, K. (2007) 'Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model', *Nat Neurosci,* 10(5), pp. 608-14.

Dibner, C., Schibler, U. and Albrecht, U. (2010) 'The mammalian circadian timing system: organization and coordination of central and peripheral clocks', *Annu Rev Physiol,* 72, pp. 517-49.

Dickson, C. T. and Alonso, A. (1997) 'Muscarinic induction of synchronous population activity in the entorhinal cortex', *J Neurosci,* 17(17), pp. 6729-44.

Dickson, D. W., Lee, S. C., Mattiace, L. A., Yen, S. H. and Brosnan, C. (1993) 'Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease', *Glia,* 7(1), pp. 75-83.

Dillon, S. E., Tsivos, D., Knight, M., McCann, B., Pennington, C., Shiel, A. I., Conway, M. E., Newson, M. A., Kauppinen, R. A. and Coulthard, E. J. (2017) 'The impact of ageing reveals distinct roles for human dentate gyrus and CA3 in pattern separation and object recognition memory', *Scientific Reports,* 7.

Diógenes, M. J., Costenla, A. R., Lopes, L. V., Jerónimo-Santos, A., Sousa, V. C., Fontinha, B. M., Ribeiro, J. A. and Sebastião, A. M. (2011) 'Enhancement of LTP in aged rats is dependent on endogenous BDNF', *Neuropsychopharmacology,* 36(9), pp. 1823-36.

Dong, H. W., Petrovich, G. D. and Swanson, L. W. (2001) 'Topography of projections from amygdala to bed nuclei of the stria terminalis', *Brain Research Reviews,* 38(1-2), pp. 192-246.

Dotan, I., Werner, L., Vigodman, S., Weiss, S., Brazowski, E., Maharshak, N., Chen, O., Tulchinsky, H., Halpern, Z. and Guzner-Gur, H. (2010) 'CXCL12 Is a Constitutive and Inflammatory Chemokine in the Intestinal Immune System', *Inflammatory Bowel Diseases,* 16(4), pp. 583-592.

Driscoll, I., Hamilton, D. A., Petropoulos, H., Yeo, R. A., Brooks, W. M., Baumgartner, R. N. and Sutherland, R. J. (2003) 'The aging hippocampus: Cognitive, biochemical and structural findings', *Cerebral Cortex,* 13(12), pp. 1344-1351.

Driscoll, I., Howard, S. R., Stone, J. C., Monfils, M. H., Tomanek, B., Brooks, W. M. and Sutherland, R. J. (2006) 'The aging hippocampus: A multi-level analysis in the rat', *Neuroscience,* 139(4), pp. 1173-1185.

Duan, L. H., Zhang, X. D., Miao, W. Y., Sun, Y. J., Xiong, G. L., Wu, Q. Z., Li, G. Y., Yang, P., Yu, H., Li, H. M. Z., Wang, Y., Zhang, M., Hu, L. Y., Tong, X., Zhou, W. H. and Yu, X. (2018) 'PDGFR beta Cells Rapidly Relay Inflammatory Signal from the Circulatory System to Neurons via Chemokine CCL2', *Neuron,* 100(1), pp. 183-+.

Dzenko, K. A., Song, L., Ge, S. J., Kuziel, W. A. and Pachter, J. S. (2005) 'CCR2 expression by brain microvascular endothelial cells is critical for macrophage transendothelial migration in response to CCL2', *Microvascular Research,* 70(1-2), pp. 53-64.

Edderkaoui, B., Sargsyan, L., Hetrick, A. and Li, H. (2018) 'Deficiency of Duffy Antigen Receptor for Chemokines Ameliorated Cochlear Damage From Noise Exposure', *Front Mol Neurosci,* 11, pp. 173.

Eeckman, F. H. and Freeman, W. J. (1990) 'CORRELATIONS BETWEEN UNIT FIRING AND EEG IN THE RAT OLFACTORY SYSTEM', *Brain Research,* 528(2), pp. 238-244.

Eichenbaum, H. (2000) 'A cortical-hippocampal system for declarative memory', *Nature Reviews Neuroscience,* 1(1), pp. 41-50.

Eichenbaum, H. and Fortin, N. J. (2005) 'Bridging the gap between brain and behavior: Cognitive and neural mechanisms of episodic memory', *Journal of the Experimental Analysis of Behavior,* 84(3), pp. 619-629.

Eisenberger, N. I., Gable, S. L. and Lieberman, M. D. (2007) 'Functional magnetic resonance imaging responses relate to differences in real-world social experience', *Emotion,* 7(4), pp. 745-54.

Ek, M., Kurosawa, M., Lundeberg, T. and Ericsson, A. (1998) 'Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins', *J Neurosci,* 18(22), pp. 9471-9.

Ekdahl, C. T., Claasen, J. H., Bonde, S., Kokaia, Z. and Lindvall, O. (2003) 'Inflammation is detrimental for neurogenesis in adult brain', *Proceedings of the National Academy of Sciences of the United States of America,* 100(23), pp. 13632-13637.

Engel, A. K., Fries, P. and Singer, W. (2001) 'Dynamic predictions: oscillations and synchrony in top-down processing', *Nat Rev Neurosci,* 2(10), pp. 704-16.

Engelhardt, J. I. and Appel, S. H. (1990) 'IgG reactivity in the spinal cord and motor cortex in amyotrophic lateral sclerosis', *Arch Neurol,* 47(11), pp. 1210-6.

Epstein, I. and Finkbeiner, S. (2018) 'The Arc of cognition: Signaling cascades regulating Arc and implications for cognitive function and disease', *Seminars in Cell & Developmental Biology,* 77, pp. 63-72.

Ermentrout, G. B. and Kopell, N. (1998) 'Fine structure of neural spiking and synchronization in the presence of conduction delays', *Proc Natl Acad Sci U S A,* 95(3), pp. 1259-64.

Ernyey, A. J., Grohmann Pereira, T., Kozma, K., Kouhnavardi, S., Kassai, F. and Gyertyán, I. (2019) 'Following of aging process in a new motor skill learning model, "pot jumping" in rats', *Geroscience,* 41(3), pp. 309-319.

Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D., Franceschi, C. and Paganelli, R. (1993) 'Increased cytokine production in mononuclear cells of healthy elderly people', *Eur J Immunol,* 23(9), pp. 2375-8.

Fanselow, M. S. and Dong, H. W. (2010) 'Are the dorsal and ventral hippocampus functionally distinct structures?', *Neuron,* 65(1), pp. 7-19.

Fell, J., Klaver, P., Lehnertz, K., Grunwald, T., Schaller, C., Elger, C. E. and Fernandez, G. (2001) 'Human memory formation is accompanied by rhinal-hippocampal coupling and decoupling', *Nature Neuroscience,* 4(12), pp. 1259-1264..

Feng, T., Alicea, C., Pham, V., Kirk, A. and Pieraut, S. (2021) 'Experience-Dependent Inhibitory Plasticity Is Mediated by CCK plus Basket Cells in the Developing Dentate Gyrus', *Journal of Neuroscience,* 41(21), pp. 4607-4619.

Feng, X. Y., Guo, J., Sigmon, H. C., Sloan, R. P., Brickman, A. M., Provenzano, F. A., Small, S. A. and Alzheimers Dis Neuroimaging, I. (2020) 'Brain regions vulnerable and resistant to aging without Alzheimer's disease', *Plos One,* 15(7).

Ferbinteanu, J., Holsinger, R. M. D. and McDonald, R. J. (1999) 'Lesions of the medial or lateral perforant path have different effects on hippocampal contributions to place learning and on fear conditioning to context', *Behavioural Brain Research,* 101(1), pp. 65-84.

Fernandez, G., Effern, A., Grunwald, T., Pezer, N., Lehnertz, K., Dumpelmann, M., Van Roost, D. and Elger, C. E. (1999) 'Real-time tracking of memory formation in the human rhinal cortex and hippocampus', *Science,* 285(5433), pp. 1582-1585.

Fernandez-Arjona, M. D., Grondona, J. M., Granados-Duran, P., Fernandez-Llebrez, P. and Lopez-Avalos, M. D. (2017) 'Microglia Morphological Categorization in a Rat Model of Neuroinflammation by Hierarchical Cluster and Principal Components Analysis', *Frontiers in Cellular Neuroscience,* 11.

Fernandez-Ruiz, A., Oliva, A., Soula, M., Rocha-Almeida, F., Nagy, G. A., Martin-Vazquez, G. and Buzsaki, G. (2021) 'Gamma rhythm communication between entorhinal cortex and dentate gyrus neuronal assemblies', *Science,* 372(6537), pp. 50-+.

Ferrucci, L. and Fabbri, E. (2018) 'Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty', *Nature Reviews Cardiology,* 15(9), pp. 505-522.

Ferrucci, L., Semba, R. D., Guralnik, J. M., Ershler, W. B., Bandinelli, S., Patel, K. V., Sun, K., Woodman, R. C., Andrews, N. C., Cotter, R. J., Ganz, T., Nemeth, E. and Longo, D. L. (2010) 'Proinflammatory state, hepcidin, and anemia in older persons', *Blood,* 115(18), pp. 3810-3816.

Fifková, E. (1975) 'Two types of terminal degeneration in the molecular layer of the dentate fascia following lesions of the entorhinal cortex', *Brain Res,* 96(1), pp. 169-75.

Finch, C. E. and Crimmins, E. M. (2004) 'Inflammatory exposure and historical changes in human life-spans', *Science,* 305(5691), pp. 1736-9.

Fisahn, A., Contractor, A., Traub, R. D., Buhl, E. H., Heinemann, S. F. and McBain, C. J. (2004) 'Distinct roles for the kainate receptor subunits GluR5 and GluR6 in kainate-induced hippocampal gamma oscillations', *J Neurosci,* 24(43), pp. 9658-68.

Fisahn, A., Pike, F. G., Buhl, E. H. and Paulsen, O. (1998) 'Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro', *Nature,* 394(6689), pp. 186-9.

Fleming, R. L., Wilson, W. A. and Swartzwelder, H. S. (2007) 'Magnitude and ethanol sensitivity of tonic GABA(A) receptor-mediated inhibition in dentate gyrus changes from adolescence to adulthood', *Journal of Neurophysiology,* 97(5), pp. 3806-3811.

Foresti, M. L., Arisi, G. M., Katki, K., Montanez, A., Sanchez, R. M. and Shapiro, L. A. (2009) 'Chemokine CCL2 and its receptor CCR2 are increased in the hippocampus following pilocarpine-induced status epilepticus', *Journal of Neuroinflammation,* 6, pp. 11.

Foster, J. R. (2001) 'The functions of cytokines and their uses in toxicology', *Int J Exp Pathol,* 82(3), pp. 171-92.

Foster, T. C., Barnes, C. A., Rao, G. and McNaughton, B. L. (1991) 'Increase in perforant path quantal size in aged F-344 rats', *Neurobiol Aging,* 12(5), pp. 441-8.

Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E. and De Benedictis, G. (2000) 'Inflamm-aging - An evolutionary perspective on immunosenescence', *Molecular and Cellular Gerontology,* 908, pp. 244-254.

Franceschi, C. and Campisi, J. (2014) 'Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases', *Journals of Gerontology Series a-Biological Sciences and Medical Sciences,* 69, pp. S4-S9.

Francia, N., Cirulli, F., Chiarotti, F., Antonelli, A., Aloe, L. and Alleva, E. (2006) 'Spatial memory deficits in middle-aged mice correlate with lower exploratory activity and a subordinate status: role of hippocampal neurotrophins', *European Journal of Neuroscience,* 23(3), pp. 711-728.

French, C. R., Sah, P., Buckett, K. J. and Gage, P. W. (1990) 'A voltage-dependent persistent sodium current in mammalian hippocampal neurons', *J Gen Physiol,* 95(6), pp. 1139-57.

Freund, T. F. and Buzsaki, G. (1996) 'Interneurons of the hippocampus', *Hippocampus,* 6(4), pp. 347-470.

Frey, B. N., Andreazza, A. C., Nery, F. G., Martins, M. R., Quevedo, J., Soares, J. C. and Kapczinski, F. (2007) 'The role of hippocampus in the pathophysiology of bipolar disorder', *Behavioural Pharmacology,* 18(5-6), pp. 419-430.

Fries, P. (2009) 'Neuronal gamma-band synchronization as a fundamental process in cortical computation', *Annu Rev Neurosci,* 32, pp. 209-24.

Froc, D. J., Eadie, B., Li, A. M., Wodtke, K., Tse, M. and Christie, B. R. (2003) 'Reduced synaptic plasticity in the lateral perforant path input to the dentate gyrus of aged C57BL/6 mice', *Journal of Neurophysiology,* 90(1), pp. 32-38.

Frotscher, M., Zhao, S. and Forster, E. (2007) 'Development of cell and fiber layers in the dentate gyrus', *Dentate Gyrus: a Comphrehensive Guide to Structure, Function, and Clinical Implications,* 163, pp. 133-+.

Fu, Y., Yu, Y., Paxinos, G., Watson, C. and Rusznák, Z. (2015) 'Aging-dependent changes in the cellular composition of the mouse brain and spinal cord', *Neuroscience,* 290, pp. 406-20.

Fukuma, N., Akimitsu, N., Hamamoto, H., Kusuhara, H., Sugiyama, Y. and Sekimizu, K. (2003) 'A role of the Duffy antigen for the maintenance of plasma chemokine concentrations', *Biochemical and Biophysical Research Communications,* 303(1), pp. 137-139.

Fulop, T., Larbi, A., Douziech, N., Fortin, C., Guerard, K. P., Lesur, O., Khalil, A. and Dupuis, G. (2004) 'Signal transduction and functional changes in neutrophils with aging', *Aging Cell,* 3(4), pp. 217-226.

Fusar-Poli, P., Placentino, A., Carletti, F., Landi, P., Allen, P., Surguladze, S., Benedetti, F., Abbamonte, M., Gasparotti, R., Barale, F., Perez, J., McGuire, P. and Politi, P. (2009) 'Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies', *J Psychiatry Neurosci,* 34(6), pp. 418-32.

Fyhn, M., Molden, S., Witter, M. P., Moser, E. I. and Moser, M. B. (2004) 'Spatial representation in the entorhinal cortex', *Science,* 305(5688), pp. 1258-1264.

Förster, R., Davalos-Misslitz, A. C. and Rot, A. (2008) 'CCR7 and its ligands: balancing immunity and tolerance', *Nat Rev Immunol,* 8(5), pp. 362-71.

Gabrieli, J. D. E., Brewer, J. B., Desmond, J. E. and Glover, G. H. (1997) 'Separate neural bases of two fundamental memory processes in the human medial temporal lobe', *Science,* 276(5310), pp. 264-266.

Gage, F. H., Björklund, A., Stenevi, U., Dunnett, S. B. and Kelly, P. A. (1984) 'Intrahippocampal septal grafts ameliorate learning impairments in aged rats', *Science,* 225(4661), pp. 533-6.

Gage, F. H. and Thompson, R. G. (1980) 'Differential distribution of norepinephrine and serotonin along the dorsal-ventral axis of the hippocampal formation', *Brain Res Bull,* 5(6), pp. 771-3.

Galloway, A. (1993) 'THE EVOLUTIONARY BIOLOGY OF AGING - ROSE,MR', *American Journal of Physical Anthropology,* 91(2), pp. 260-262.

Gao, Y. J., Zhang, L., Samad, O. A., Suter, M. R., Yasuhiko, K., Xu, Z. Z., Park, J. Y., Lind, A. L., Ma, Q. and Ji, R. R. (2009) 'JNK-Induced MCP-1 Production in Spinal Cord Astrocytes Contributes to Central Sensitization and Neuropathic Pain', *Journal of Neuroscience,* 29(13), pp. 4096-4108.

Garden, G. A. (2002) 'Microglia in human immunodeficiency virus-associated neurodegeneration', *Glia,* 40(2), pp. 240-251.

Gardner, L., Patterson, A. M., Ashton, B. A., Stone, M. A. and Middleton, J. (2004) 'The human Duffy antigen binds selected inflammatory but not homeostatic chemokines', *Biochem Biophys Res Commun,* 321(2), pp. 306-12.

Garg, S. K., Delaney, C., Shi, H. and Yung, R. (2014) 'Changes in adipose tissue macrophages and T cells during aging', *Crit Rev Immunol,* 34(1), pp. 1-14.

Ge, S. and Pachter, J. S. (2004) 'Caveolin-1 knockdown by small interfering RNA suppresses responses to the chemokine monocyte chemoattractant protein-1 by human astrocytes', *J Biol Chem,* 279(8), pp. 6688-95.

Geinisman, Y., deToledo-Morrell, L., Morrell, F., Persina, I. S. and Rossi, M. (1992) 'Age-related loss of axospinous synapses formed by two afferent systems in the rat dentate gyrus as revealed by the unbiased stereological dissector technique', *Hippocampus,* 2(4), pp. 437-44.

Geleff, S., Draganovici, D., Jaksch, P. and Segerer, S. (2010) 'The role of chemokine receptors in acute lung allograft rejection', *European Respiratory Journal,* 35(1), pp. 167-175.

Gendelman, H. E. (2002) 'Neural immunity: Friend or foe?', *Journal of Neurovirology,* 8(6), pp. 474-479.

Gerli, R., Monti, D., Bistoni, O., Mazzone, A. M., Peri, G., Cossarizza, A., Di Gioacchino, M., Cesarotti, M. E. F., Doni, A., Mantovani, A., Franceschi, C. and Paganelli, R. (2000) 'Chemokines, sTNF-Rs and sCD30 serum levels in healthy aged people and centenarians', *Mechanisms of Ageing and Development,* 121(1-3), pp. 37-46.

Geyer, S., Jacobs, M. and Hsu, N. J. (2019) 'Immunity Against Bacterial Infection of the Central Nervous System: An Astrocyte Perspective', *Front Mol Neurosci,* 12, pp. 57.

Gibertini, M., Newton, C., Friedman, H. and Klein, T. W. (1995) 'Spatial-learning impairment in mice infected with legionella-pneumophila or administered exogenous interleukin-1-beta', *Brain Behavior and Immunity,* 9(2), pp. 113-128.

Gilbert, P. E. and Brushfield, A. M. (2009) 'The role of the CA3 hippocampal subregion in spatial memory: A process oriented behavioral assessment', *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 33(5), pp. 774-781.

Ginhoux, F., Lim, S., Hoeffel, G., Low, D. and Huber, T. (2013) 'Origin and differentiation of microglia', *Frontiers in Cellular Neuroscience,* 7.

Glabinski, A. R., Balasingam, V., Tani, M., Kunkel, S. L., Strieter, R. M., Yong, V. W. and Ransohoff, R. M. (1996) 'Chemokine monocyte chemoattractant protein-1 is expressed by astrocytes after mechanical injury to the brain', *Journal of Immunology,* 156(11), pp. 4363-4368.

Glabinski, A. R. and Ransohoff, R. M. (1999) 'Chemokines and chemokine receptors in CNS pathology', *Journal of Neurovirology,* 5(1), pp. 3-12.

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C. and Gage, F. H. (2010) 'Mechanisms underlying inflammation in neurodegeneration', *Cell,* 140(6), pp. 918-34.

Glezeva, N. and Baugh, J. A. (2014) 'Role of inflammation in the pathogenesis of heart failure with preserved ejection fraction and its potential as a therapeutic target', *Heart Fail Rev,* 19(5), pp. 681-94.

Gloveli, T., Egorov, A. V., Schmitz, D., Heinemann, U. and Müller, W. (1999) 'Carbachol-induced changes in excitability and [Ca2+]i signalling in projection cells of medial entorhinal cortex layers II and III', *Eur J Neurosci,* 11(10), pp. 3626-36.

Godbout, J. P., Chen, J., Abraham, J., Richwine, A. F., Berg, B. M., Kelley, K. W. and Johnson, R. W. (2005) 'Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system', *Faseb j,* 19(10), pp. 1329-31.

Gomez, C. R., Hirano, S., Cutro, B. T., Birjandi, S., Baila, H., Nomellini, V. and Kovacs, E. J. (2007) 'Advanced age exacerbates the pulmonary inflammatory response after lipopolysaccharide exposure', *Crit Care Med,* 35(1), pp. 246-51.

Gomez-Nicola, D. and Perry, V. H. (2015) 'Microglial dynamics and role in the healthy and diseased brain: a paradigm of functional plasticity', *Neuroscientist,* 21(2), pp. 169-84.

GomezIsla, T., Price, J. L., McKeel, D. W., Morris, J. C., Growdon, J. H. and Hyman, B. T. (1996) 'Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease', *Journal of Neuroscience,* 16(14), pp. 4491-4500.

Goodman, M. S., Kumar, S., Zomorrodi, R., Ghazala, Z., Cheam, A. S. M., Barr, M. S., Daskalakis, Z. J., Blumberger, D. M., Fischer, C., Flint, A., Mah, L., Herrmann, N., Bowie, C. R., Mulsant, B. H. and Rajji, T. K. (2018) 'Theta-Gamma Coupling and Working Memory in Alzheimer's Dementia and Mild Cognitive Impairment', *Front Aging Neurosci,* 10, pp. 101.

Goshen, I. and Yirmiya, R. (2009) 'Interleukin-1 (IL-1): A central regulator of stress responses', *Frontiers in Neuroendocrinology,* 30(1), pp. 30-45.

Gosselin, R. D., Varela, C., Banisadr, G., Mechighel, P., Rostene, W., Kitabgi, P. and Melik-Parsadaniantz, S. (2005) 'Constitutive expression of CCR2 chemokine receptor and inhibition by MCP-1/CCL2 of GABA-induced currents in spinal cord neurones', *Journal of Neurochemistry,* 95(4), pp. 1023-1034.

Gothard, K. M., Skaggs, W. E., Moore, K. M. and McNaughton, B. L. (1996) 'Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task', *J Neurosci,* 16(2), pp. 823-35.

Gray, C. M. and Singer, W. (1989) 'Stimulus-specific neuronal oscillations in orientation columns of cat visual cortex', *Proc Natl Acad Sci U S A,* 86(5), pp. 1698-702.

Green, J. D. and Arduini, A. A. (1954) 'Hippocampal electrical activity in arousal', *J Neurophysiol,* 17(6), pp. 533-57.

Greene, P., Howard, M., Bhattacharyya, R. and Fellous, J. M. (2013) 'Hippocampal Anatomy Supports the Use of Context in Object Recognition: A Computational Model', *Computational Intelligence and Neuroscience*, pp. 19.

Grundman, M., Sencakova, D., Jack, C. R., Jr., Petersen, R. C., Kim, H. T., Schultz, A., Weiner, M. F., DeCarli, C., DeKosky, S. T., van Dyck, C., Thomas, R. G. and Thal, L. J. (2002) 'Brain MRI hippocampal volume and prediction of clinical status in a mild cognitive impairment trial', *J Mol Neurosci,* 19(1-2), pp. 23-7.

Gruol, D. L., Vo, K. and Bray, J. G. (2014) 'Increased astrocyte expression of IL-6 or CCL2 in transgenic mice alters levels of hippocampal and cerebellar proteins', *Frontiers in Cellular Neuroscience,* 8.

Gruol, D. L., Vo, K., Bray, J. G. and Roberts, A. J. (2014) 'CCL2-ethanol interactions and hippocampal synaptic protein expression in a transgenic mouse model', *Front Integr Neurosci,* 8, pp. 29.

Gupta, A. S., van der Meer, M. A. A., Touretzky, D. S. and Redish, A. D. (2012) 'Segmentation of spatial experience by hippocampal theta sequences', *Nature Neuroscience,* 15(7), pp. 1032-1039.

Gureviciene, I., Gurevicius, K. and Tanila, H. (2009) 'Aging and alpha-synuclein affect synaptic plasticity in the dentate gyrus', *Journal of Neural Transmission,* 116(1), pp. 13-22.

Gutierrez, E. G., Banks, W. A. and Kastin, A. J. (1993) 'MURINE TUMOR-NECROSIS-FACTOR-ALPHA IS TRANSPORTED FROM BLOOD TO BRAIN IN THE MOUSE', *Journal of Neuroimmunology,* 47(2), pp. 169-176.

Guyon, A., Skrzydelski, D., De Giry, I., Rovère, C., Conductier, G., Trocello, J. M., Daugé, V., Kitabgi, P., Rostène, W., Nahon, J. L. and Mélik Parsadaniantz, S. (2009) 'Long term exposure to the chemokine CCL2 activates the nigrostriatal dopamine system: a novel mechanism for the control of dopamine release', *Neuroscience,* 162(4), pp. 1072-80.

Gyoneva, S. and Ransohoff, R. M. (2015) 'Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell-cell communication by chemokines', *Trends in Pharmacological Sciences,* 36(7), pp. 471-480.

Gómez-Nicola, D., Fransen, N. L., Suzzi, S. and Perry, V. H. (2013) 'Regulation of microglial proliferation during chronic neurodegeneration', *J Neurosci,* 33(6), pp. 2481-93.

Habib, N., McCabe, C., Medina, S., Varshavsky, M., Kitsberg, D., Dvir-Szternfeld, R., Green, G., Dionne, D., Nguyen, L., Marshall, J. L., Chen, F., Zhang, F., Kaplan, T., Regev, A. and Schwartz, M. (2020) 'Disease-associated astrocytes in Alzheimer's disease and aging', *Nat Neurosci,* 23(6), pp. 701-706.

Hadley, T. J. and Peiper, S. C. (1997) 'From malaria to chemokine receptor: The emerging physiologic role of the duffy blood group antigen', *Blood,* 89(9), pp. 3077-3091.

Hafting, T., Fyhn, M., Molden, S., Moser, M. B. and Moser, E. I. (2005) 'Microstructure of a spatial map in the entorhinal cortex', *Nature,* 436(7052), pp. 801-806.

Haga, S., Akai, K. and Ishii, T. (1989) 'Demonstration of microglial cells in and around senile (neuritic) plaques in the Alzheimer brain. An immunohistochemical study using a novel monoclonal antibody', *Acta Neuropathol,* 77(6), pp. 569-75.

Hansell, C. A., Hurson, C. E. and Nibbs, R. J. (2011) 'DARC and D6: silent partners in chemokine regulation?', *Immunol Cell Biol,* 89(2), pp. 197-206.

Hansen, L. A., Armstrong, D. M. and Terry, R. D. (1987) 'An immunohistochemical quantification of fibrous astrocytes in the aging human cerebral cortex', *Neurobiol Aging,* 8(1), pp. 1-6.

Hara, H., Onodera, H., Kogure, K. and Akaike, N. (1990) 'The regional difference of neuronal susceptibility in the dentate gyrus to hypoxia', *Neuroscience Letters,* 115(2-3), pp. 189-194.

Hargreaves, E. L., Rao, G., Lee, I. and Knierim, J. J. (2005) 'Major dissociation between medial and lateral entorhinal input to dorsal hippocampus', *Science,* 308(5729), pp. 1792-1794.

Haring, J. H. and Davis, J. N. (1985) 'Differential distribution of locus coeruleus projections to the hippocampal formation: anatomical and biochemical evidence', *Brain Res,* 325(1-2), pp. 366-9.

Harker, K. T. and Whishaw, I. Q. (2004) 'Impaired place navigation in place and matching-to-place swimming pool tasks follows both retrosplenial cortex lesions and cingulum bundle lesions in rats', *Hippocampus,* 14(2), pp. 224-31.

Harrington, J. R. (2000) 'The role of MCP-1 in atherosclerosis', *Stem Cells,* 18(1), pp. 65-66.

Harris, M. G., Hulseberg, P., Ling, C. Y., Karman, J., Clarkson, B. D., Harding, J. S., Zhang, M. X., Sandor, A., Christensen, K., Nagy, A., Sandor, M. and Fabry, Z. (2014) 'Immune privilege of the CNS is not the consequence of limited antigen sampling', *Scientific Reports,* 4.

Hasty, P. and Vijg, J. (2004) 'Accelerating aging by mouse reverse genetics: a rational approach to understanding longevity', *Aging Cell,* 3(2), pp. 55-65.

Hattiangady, B., Rao, M. S., Shetty, G. A. and Shetty, A. K. (2005) 'Brain-derived neurotrophic factor, phosphorylated cyclic AMP response element binding protein and neuropeptide Y decline as early as middle age in the dentate gyrus and CA1 and CA3 subfields of the hippocampus', *Experimental Neurology,* 195(2), pp. 353-371.

Hayman, R. M., Chakraborty, S., Anderson, M. I. and Jeffery, K. J. (2003) 'Context-specific acquisition of location discrimination by hippocampal place cells', *Eur J Neurosci,* 18(10), pp. 2825-34.

He, M. F., Dong, H. Q., Huang, Y. H., Lu, S. M., Zhang, S., Qian, Y. N. and Jin, W. J. (2016) 'Astrocyte-Derived CCL2 is Associated with M1 Activation and Recruitment of Cultured Microglial Cells', *Cellular Physiology and Biochemistry,* 38(3), pp. 859-870.

Hefft, S. and Jonas, P. (2005) 'Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse', *Nat Neurosci,* 8(10), pp. 1319-28.

Heneka, M. T., Carson, M. J., El Khoury, J., Landreth, G. E., Brosseron, F., Feinstein, D. L., Jacobs, A. H., Wyss-Coray, T., Vitorica, J., Ransohoff, R. M., Herrup, K., Frautschy, S. A., Finsen, B., Brown, G. C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G. C., Town, T., Morgan, D., Shinohara, M. L., Perry, V. H., Holmes, C., Bazan, N. G., Brooks, D. J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C. A., Breitner, J. C., Cole, G. M., Golenbock, D. T. and Kummer, M. P. (2015) 'Neuroinflammation in Alzheimer's disease', *Lancet Neurol,* 14(4), pp. 388-405.

Henke, P. G. (1990) 'Hippocampal pathway to the amygdala and stress ulcer development', *Brain Res Bull,* 25(5), pp. 691-5.

Herculano-Houzel, S., Munk, M. H., Neuenschwander, S. and Singer, W. (1999) 'Precisely synchronized oscillatory firing patterns require electroencephalographic activation', *J Neurosci,* 19(10), pp. 3992-4010.

Herman, J. P., Ostrander, M. M., Mueller, N. K. and Figueiredo, H. (2005a) 'Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis', *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 29(8), pp. 1201-1213.

Hinojosa, A. E., Garcia-Bueno, B., Leza, J. C. and Madrigal, J. L. (2011a) 'CCL2/MCP-1 modulation of microglial activation and proliferation', *J Neuroinflammation,* 8, pp. 77.

Hirsch, E. C., Vyas, S. and Hunot, S. (2012) 'Neuroinflammation in Parkinson's disease', *Parkinsonism Relat Disord,* 18 Suppl 1, pp. S210-2.

Hollup, S. A., Kjelstrup, K. G., Hoff, J., Moser, M. B. and Moser, E. I. (2001) 'Impaired recognition of the goal location during spatial navigation in rats with hippocampal lesions', *J Neurosci,* 21(12), pp. 4505-13.

Holmes, C. (2013) 'Review: Systemic inflammation and Alzheimer's disease', *Neuropathology and Applied Neurobiology,* 39(1), pp. 51-68.

Hormuzdi, S. G., Pais, I., LeBeau, F. E., Towers, S. K., Rozov, A., Buhl, E. H., Whittington, M. A. and Monyer, H. (2001) 'Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice', *Neuron,* 31(3), pp. 487-95.

Horuk, R., Martin, A., Hesselgesser, J., Hadley, T., Lu, Z. H., Wang, Z. X. and Peiper, S. C. (1996) 'The Duffy antigen receptor for chemokines: Structural analysis and expression in the brain', *Journal of Leukocyte Biology,* 59(1), pp. 29-38.

Horuk, R., Martin, A. W., Wang, Z., Schweitzer, L., Gerassimides, A., Guo, H., Lu, Z., Hesselgesser, J., Perez, H. D., Kim, J., Parker, J., Hadley, T. J. and Peiper, S. C. (1997) 'Expression of chemokine receptors by subsets of neurons in the central nervous system', *J Immunol,* 158(6), pp. 2882-90.

Hotamisligil, G. S. (2006) 'Inflammation and metabolic disorders', *Nature,* 444(7121), pp. 860-7.

Huang, C. C. and Hsu, K. S. (2003) 'Reexamination of the role of hyperpolarization-activated cation channels in short- and long-term plasticity at hippocampal mossy fiber synapses', *Neuropharmacology,* 44(7), pp. 968-981.

Hudson, J. A., Mayhew, E. M. and Prabhakar, J. (2011) 'The development of episodic foresight: emerging concepts and methods', *Adv Child Dev Behav,* 40, pp. 95-137.

Hughes, C. E. and Nibbs, R. J. B. (2018) 'A guide to chemokines and their receptors', *Febs j,* 285(16), pp. 2944-2971.

Hughes, R. N. (2004) 'The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory', *Neuroscience and Biobehavioral Reviews,* 28(5), pp. 497-505.

Huxter, J. R., Miranda, J. A. and Dias, R. (2012) 'The hippocampal physiology of approaching middle-age: Early indicators of change', *Hippocampus,* 22(9), pp. 1923-1940.

Hyman, B. T., Van Hoesen, G. W., Damasio, A. R. and Barnes, C. L. (1984) 'Alzheimer's disease: cell-specific pathology isolates the hippocampal formation', *Science,* 225(4667), pp. 1168-70.

Hyman, B. T., Van Hoesen, G. W., Kromer, L. J. and Damasio, A. R. (1986) 'Perforant pathway changes and the memory impairment of Alzheimer's disease', *Ann Neurol,* 20(4), pp. 472-81.

Inadera, H., Egashira, K., Takemoto, M., Ouchi, Y. and Matsushima, K. (1999) 'Increase in circulating levels of monocyte chemoattractant protein-1 with aging', *J Interferon Cytokine Res,* 19(10), pp. 1179-82.

Insausti, R., Herrero, M. T. and Witter, M. P. (1997) 'Entorhinal cortex of the rat: Cytoarchitectonic subdivisions and the origin and distribution of cortical efferents', *Hippocampus,* 7(2), pp. 146-183.

Ishizuka, K., Kimura, T., Igata-yi, R., Katsuragi, S., Takamatsu, J. and Miyakawa, T. (1997a) 'Identification of monocyte chemoattractant protein-1 in senile plaques and reactive microglia of Alzheimer's disease', *Psychiatry Clin Neurosci,* 51(3), pp. 135-8.

Ishizuka, N. (2001) 'Laminar organization of the pyramidal cell layer of the subiculum in the rat', *J Comp Neurol,* 435(1), pp. 89-110.

Itagaki, S., McGeer, P. L., Akiyama, H., Zhu, S. and Selkoe, D. (1989) 'Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease', *J Neuroimmunol,* 24(3), pp. 173-82.

Iturra-Mena, A. M., Aguilar-Rivera, M., Arriagada-Solimano, M., Perez-Valenzuela, C., Fuentealba, P. and Dagnino-Subiabre, A. (2019) 'Impact of Stress on Gamma Oscillations in the Rat Nucleus Accumbens During Spontaneous Social Interaction', *Frontiers in Behavioral Neuroscience,* 13.

Iwasaki, A. and Medzhitov, R. (2015) 'Control of adaptive immunity by the innate immune system', *Nature Immunology,* 16(4), pp. 343-353.

Jack, C. R. and Holtzman, D. M. (2013) 'Biomarker Modeling of Alzheimer's Disease', *Neuron,* 80(6), pp. 1347-1358.

Jacobson, L. and Sapolsky, R. (1991) 'The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis', *Endocr Rev,* 12(2), pp. 118-34.

Jacobson, T. K., Howe, M. D., Schmidt, B., Hinman, J. R., Escabi, M. A. and Markus, E. J. (2013) 'Hippocampal theta, gamma, and theta-gamma coupling: effects of aging, environmental change, and cholinergic activation', *Journal of Neurophysiology,* 109(7), pp. 1852-1865.

Jagadeesh, B., Gray, C. M. and Ferster, D. (1992) 'Visually evoked oscillations of membrane potential in cells of cat visual cortex', *Science,* 257(5069), pp. 552-4.

Jarrard, L. E. (1978) 'Selective hippocampal lesions: differential effects on performance by rats of a spatial task with preoperative versus postoperative training', *J Comp Physiol Psychol,* 92(6), pp. 1119-27.

Jensen, C. J., Massie, A. and De Keyser, J. (2013) 'Immune Players in the CNS: The Astrocyte', *Journal of Neuroimmune Pharmacology,* 8(4), pp. 824-839.

Jiang, Y. Z., Chen, Y. W., Huang, C. L., Xia, A. Q., Wang, G. H. and Liu, S. (2021) 'Hyperbaric oxygen therapy improves neurological function via the p38-MAPK/CCL2 signaling pathway following traumatic brain injury', *Neuroreport,* 32(15), pp. 1255-1262.

Jilma-Stohlawetz, P., Homoncik, M., Drucker, C., Marsik, C., Rot, A., Mayr, W. R., Seibold, B. and Jilma, B. (2001a) 'Fy phenotype and gender determine plasma levels of monocyte chemotactic protein', *Transfusion,* 41(3), pp. 378-381.

Jinno, S., Ishizuka, S. and Kosaka, T. (2003) 'Ionic currents underlying rhythmic bursting of ventral mossy cells in the developing mouse dentate gyrus', *European Journal of Neuroscience,* 17(7), pp. 1338-1354.

Jones, M. W., Errington, M. L., French, P. J., Fine, A., Bliss, T. V., Garel, S., Charnay, P., Bozon, B., Laroche, S. and Davis, S. (2001) 'A requirement for the immediate early

gene Zif268 in the expression of late LTP and long-term memories', *Nat Neurosci,* 4(3), pp. 289-96.

Joëls, M. (2001) 'Corticosteroid actions in the hippocampus', *J Neuroendocrinol,* 13(8), pp. 657-69.

Joëls, M., Karst, H., Krugers, H. J. and Lucassen, P. J. (2007) 'Chronic stress: implications for neuronal morphology, function and neurogenesis', *Front Neuroendocrinol,* 28(2-3), pp. 72-96.

Jucker, M., Walker, L. C., Kuo, H., Tian, M. and Ingram, D. K. (1994) 'Age-related fibrillar deposits in brains of C57BL/6 mice. A review of localization, staining characteristics, and strain specificity', *Mol Neurobiol,* 9(1-3), pp. 125-33.

Jung, H., Toth, P. T., White, F. A. and Miller, R. J. (2008) 'Monocyte chemoattractant protein-1 functions as a neuromodulator in dorsal root ganglia neurons', *Journal of Neurochemistry,* 104(1), pp. 254-263.

Jung, M. W. and McNaughton, B. L. (1993) 'Spatial selectivity of unit activity in the hippocampal granular layer', *Hippocampus,* 3(2), pp. 165-82.

Jutras, M. J., Fries, P. and Buffalo, E. A. (2009) 'Gamma-Band Synchronization in the Macaque Hippocampus and Memory Formation', *Journal of Neuroscience,* 29(40), pp. 12521-12531.

Jyothi, H. J., Vidyadhara, D. J., Mahadevan, A., Philip, M., Parmar, S. K., Manohari, S. G., Shankar, S. K., Raju, T. R. and Alladi, P. A. (2015) 'Aging causes morphological alterations in astrocytes and microglia in human substantia nigra pars compacta', *Neurobiol Aging,* 36(12), pp. 3321-3333.

Kany, S., Vollrath, J. T. and Relja, B. (2019) 'Cytokines in Inflammatory Disease', *Int J Mol Sci,* 20(23).

Karaca, K. G., Brito, D. V. C., Kupke, J., Zeuch, B. and Oliveira, A. M. M. (2021) 'Engram reactivation during memory retrieval predicts long-term memory performance in aged mice', *Neurobiology of Aging,* 101, pp. 256-261.

Kashiwazaki, M., Tanaka, T., Kanda, H., Ebisuno, Y., Izawa, D., Fukuma, N., Akimitsu, N., Sekimizu, K., Monden, M. and Miyasaka, M. (2003) 'A high endothelial venule-expressing promiscuous chemokine receptor DARC can bind inflammatory, but not lymphoid, chemokines and is dispensable for lymphocyte homing under physiological conditions', *Int Immunol,* 15(10), pp. 1219-27.

Kausler, D. H. (1994) *Learning and memory in normal aging. Learning and memory in normal aging.* San Diego, CA, US: Academic Press.

Kenakin, T. (2012) 'The potential for selective pharmacological therapies through biased receptor signaling', *BMC Pharmacology and Toxicology,* 13(1), pp. 3.

Kent, K., Hess, K., Tonegawa, S. and Small, S. A. (2007) 'CA3 NMDA receptors are required for experience-dependent shifts in hippocampal activity', *Hippocampus,* 17(10), pp. 1003-11.

Kerr, D. S., Campbell, L. W., Applegate, M. D., Brodish, A. and Landfield, P. W. (1991) 'Chronic stress-induced acceleration of electrophysiologic and morphometric biomarkers of hippocampal aging', *J Neurosci,* 11(5), pp. 1316-24.

Kerrén, C., Linde-Domingo, J., Hanslmayr, S. and Wimber, M. (2018) 'An Optimal Oscillatory Phase for Pattern Reactivation during Memory Retrieval', *Curr Biol,* 28(21), pp. 3383-3392.e6.

Kettenmann, H., Hanisch, U. K., Noda, M. and Verkhratsky, A. (2011) 'Physiology of microglia', *Physiol Rev,* 91(2), pp. 461-553.

Khan, U. A., Liu, L., Provenzano, F. A., Berman, D. E., Profaci, C. P., Sloan, R., Mayeux, R., Duff, K. E. and Small, S. A. (2014) 'Molecular drivers and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease', *Nature Neuroscience,* 17(2), pp. 304-311.

Kielian, T. (2014) 'Neuroinflammation: good, bad, or indifferent? PREFACE', *Journal of Neurochemistry,* 130(1), pp. 1-3.

Kim, J. J. and Fanselow, M. S. (1992) 'Modality-specific retrograde amnesia of fear', *Science,* 256(5057), pp. 675-7.

Kim, J. J., Koo, J. W., Lee, H. J. and Han, J. S. (2005) 'Amygdalar inactivation blocks stress-induced impairments in hippocampal long-term potentiation and spatial memory', *J Neurosci,* 25(6), pp. 1532-9.

Kim, J. J., Lee, H. J., Han, J. S. and Packard, M. G. (2001) 'Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning', *J Neurosci,* 21(14), pp. 5222-8.

Kishi, T., Tsumori, T., Ono, K., Yokota, S., Ishino, H. and Yasui, Y. (2000) 'Topographical organization of projections from the subiculum to the hypothalamus in the rat', *J Comp Neurol,* 419(2), pp. 205-22.

Klüver, H. and Bucy, P. C. (1937) '"Psychic blindness" and other symptoms following bilateral temporal lobectomy in Rhesus monkeys', *American Journal of Physiology,* 119, pp. 352-353.

Knierim, J. J., Neunuebel, J. P. and Deshmukh, S. S. (2014) 'Functional correlates of the lateral and medial entorhinal cortex: objects, path integration and local - global reference frames', *Philosophical Transactions of the Royal Society B-Biological Sciences,* 369(1635), pp. 7.

Kobayashi, Y. and Amaral, D. G. (2007) 'Macaque monkey retrosplenial cortex: III. Cortical efferents', *J Comp Neurol,* 502(5), pp. 810-33.

Koehl, M. and Abrous, D. N. (2011) 'A new chapter in the field of memory: adult hippocampal neurogenesis', *European Journal of Neuroscience,* 33(6), pp. 1101-1114.

Kogan, J. H., Frankland, P. W. and Silva, A. J. (2000) 'Long-term memory underlying hippocampus-dependent social recognition in mice', *Hippocampus,* 10(1), pp. 47-56.

Kohman, R. A. and Rhodes, J. S. (2013) 'Neurogenesis, inflammation and behavior', *Brain Behav Immun,* 27(1), pp. 22-32.

Kolb, B. and Muhammad, A. (2014) 'Harnessing the power of neuroplasticity for intervention', *Frontiers in Human Neuroscience,* 8, pp. 13.

Konsman, J. P., Parnet, P. and Dantzer, R. (2002) 'Cytokine-induced sickness behaviour: mechanisms and implications', *Trends Neurosci,* 25(3), pp. 154-9.

Kovacs, E. J., Palmer, J. L., Fortin, C. F., Fülöp, T., Jr., Goldstein, D. R. and Linton, P. J. (2009) 'Aging and innate immunity in the mouse: impact of intrinsic and extrinsic factors', *Trends Immunol,* 30(7), pp. 319-24.

Krabbe, K. S., Reichenberg, A., Yirmiya, R., Smed, A., Pedersen, B. K. and Bruunsgaard, H. (2005) 'Low-dose endotoxemia and human neuropsychological functions', *Brain Behav Immun,* 19(5), pp. 453-60.

Kreutzberg, G. W. (1996) 'Microglia: a sensor for pathological events in the CNS', *Trends Neurosci,* 19(8), pp. 312-8.

Ku, S. P., Tolias, A. S., Logothetis, N. K. and Goense, J. (2011) 'fMRI of the Face-Processing Network in the Ventral Temporal Lobe of Awake and Anesthetized Macaques', *Neuron,* 70(2), pp. 352-362.

Kuang, Y. N., Wu, Y. P., Jiang, H. P. and Wu, D. Q. (1996) 'Selective G protein coupling by C-C chemokine receptors', *Journal of Biological Chemistry,* 271(8), pp. 3975-3978.

Kumagai, N., Chiba, Y., Hosono, M., Fujii, M., Kawamura, N., Keino, H., Yoshikawa, K., Ishii, S., Saitoh, Y., Satoh, M., Shimada, A. and Hosokawa, M. (2007) 'Involvement of pro-inflammatory cytokines and microglia in an age-associated neurodegeneration model, the SAMP10 mouse', *Brain Research,* 1185, pp. 75-85.

Kumar, R., Clermont, G., Vodovotz, Y. and Chow, C. C. (2004) 'The dynamics of acute inflammation', *J Theor Biol,* 230(2), pp. 145-55.

Kuo, T. B. J., Li, J. Y., Hsieh, S. S. Y., Chen, J. J., Tsai, C. Y. and Yang, C. C. H. (2010) 'Effect of aging on treadmill exercise induced theta power in the rat', *Age,* 32(3), pp. 297-308.

Kurosawa, M., Shikama, Y., Furukawa, M., Arakaki, R., Ishimaru, N. and Matsushita, K. (2021) 'Chemokines Up-Regulated in Epithelial Cells Control Senescence-Associated T Cell Accumulation in Salivary Glands of Aged and Sjogren's Syndrome Model Mice', *International Journal of Molecular Sciences,* 22(5).

Köhler, C., Chan-Palay, V. and Steinbusch, H. (1981) 'The distribution and orientation of serotonin fibers in the entorhinal and other retrohippocampal areas. An immunohistochemical study with anti-serotonin antibodies in the rats brain', *Anat Embryol (Berl),* 161(3), pp. 237-64.

Lafer-Sousa, R. and Conway, B. R. (2013) 'Parallel, multi-stage processing of colors, faces and shapes in macaque inferior temporal cortex', *Nature Neuroscience,* 16(12), pp. 1870-1878.

Lai, K. S. P., Liu, C. S., Rau, A., Lanctot, K. L., Kohler, C. A., Pakosh, M., Carvalho, A. F. and Herrmann, N. (2017) 'Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies', *Journal of Neurology Neurosurgery and Psychiatry,* 88(10), pp. 876-882.

Lalo, U., Rasooli-Nejad, S. and Pankratov, Y. (2014) 'Exocytosis of gliotransmitters from cortical astrocytes: implications for synaptic plasticity and aging', *Biochem Soc Trans,* 42(5), pp. 1275-81.

Lange, C., Hemmrich, G., Klostermeier, U. C., López-Quintero, J. A., Miller, D. J., Rahn, T., Weiss, Y., Bosch, T. C. and Rosenstiel, P. (2011) 'Defining the origins of the NOD-like receptor system at the base of animal evolution', *Mol Biol Evol,* 28(5), pp. 1687-702.

Lathe, R., Sapronova, A. and Kotelevtsev, Y. (2014) 'Atherosclerosis and Alzheimer--diseases with a common cause? Inflammation, oxysterols, vasculature', *BMC Geriatr,* 14, pp. 36.

Lavenex, P., Lavenex, P. B. and Amaral, D. G. (2007) 'Spatial relational learning persists following neonatal hippocampal lesions in macaque monkeys', *Nat Neurosci,* 10(2), pp. 234-9.

Lawson, L. J., Perry, V. H., Dri, P. and Gordon, S. (1990) 'Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain', *Neuroscience,* 39(1), pp. 151-70.

Le Thuc, O., Blondeau, N., Nahon, J. L., Rovere, C. and Sci, N. Y. A. (2015) 'The complex contribution of chemokines to neuroinflammation: switching from beneficial to detrimental effects', *Neuroimmunomodulation in Health and Disease,* 1351, pp. 127-140.

Lee, C. H., Park, J. H. and Won, M. H. (2019) 'Protein expression changes of HCN1 and HCN2 in hippocampal subregions of gerbils during the normal aging process', *Iranian Journal of Basic Medical Sciences,* 22(11), pp. 1308-1313.

Lee, I., Yoganarasimha, D., Rao, G. and Knierim, J. J. (2004) 'Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3', *Nature,* 430(6998), pp. 456-9.

Lee, J. S., Frevert, C. W., Wurfel, M. M., Peiper, S. C., Wong, V. A., Ballman, K. K., Ruzinski, J. T., Rhim, J. S., Martin, T. R. and Goodman, R. B. (2003) 'Duffy antigen facilitates movement of chemokine across the endothelium in vitro and promotes neutrophil transmigration in vitro and in vivo', *J Immunol,* 170(10), pp. 5244-51.

Lee, P., Kim, J., Williams, R., Sandhir, R., Gregory, E., Brooks, W. M. and Berman, N. E. J. (2012) 'Effects of aging on blood brain barrier and matrix metalloproteases following controlled cortical impact in mice', *Experimental Neurology,* 234(1), pp. 50-61.

Lee, W. J., Liao, Y. C., Wang, Y. F., Lin, I. F., Wang, S. J. and Fuh, J. L. (2018) 'Plasma MCP-1 and Cognitive Decline in Patients with Alzheimer's Disease and Mild Cognitive Impairment: A Two-year Follow-up Study', *Scientific Reports,* 8.

Lega, B., Burke, J., Jacobs, J. and Kahana, M. J. (2016) 'Slow-Theta-to-Gamma Phase-Amplitude Coupling in Human Hippocampus Supports the Formation of New Episodic Memories', *Cerebral Cortex,* 26(1), pp. 268-278.

Lega, B. C., Jacobs, J. and Kahana, M. (2012) 'Human hippocampal theta oscillations and the formation of episodic memories', *Hippocampus,* 22(4), pp. 748-761.

Lemstra, A. W., Groen in't Woud, J. C., Hoozemans, J. J., van Haastert, E. S., Rozemuller, A. J., Eikelenboom, P. and van Gool, W. A. (2007) 'Microglia activation in sepsis: a case-control study', *J Neuroinflammation,* 4, pp. 4.

Leung, L. W. S. (1982) 'Non-linear feedback model of neuronal populations in hippocampal CA1 region', *Journal of Neurophysiology,* 47(5), pp. 845-868.

Leutgeb, J. K., Leutgeb, S., Moser, M. B. and Moser, E. I. (2007) 'Pattern separation in the dentate gyrus and CA3 of the hippocampus', *Science,* 315(5814), pp. 961-966.

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B. and Moser, E. I. (2004) 'Distinct ensemble codes in hippocampal areas CA3 and CA1', *Science,* 305(5688), pp. 1295-8.

Lewis, A. S. and Chetkovich, D. M. (2011) 'HCN channels in behavior and neurological disease: too hyper or not active enough?', *Mol Cell Neurosci,* 46(2), pp. 357-67.

Li, Q. Y. and Barres, B. A. (2018) 'Microglia and macrophages in brain homeostasis and disease', *Nature Reviews Immunology,* 18(4), pp. 225-242.

Li, T. W. and Zhu, J. H. (2019) 'Entanglement of CCR5 and Alzheimer's Disease', *Frontiers in Aging Neuroscience,* 11.

Li, X. Z., Bai, L. M., Yang, Y. P., Luo, W. F., Hu, W. D., Chen, J. P., Mao, C. J. and Liu, C. F. (2009) 'Effects of IL-6 secreted from astrocytes on the survival of dopaminergic neurons in lipopolysaccharide-induced inflammation', *Neuroscience Research,* 65(3), pp. 252-258.

Liang, B., Zhang, L., Barbera, G., Fang, W., Zhang, J., Chen, X., Chen, R., Li, Y. and Lin, D. T. (2018) 'Distinct and Dynamic ON and OFF Neural Ensembles in the Prefrontal Cortex Code Social Exploration', *Neuron,* 100(3), pp. 700-714.e9.

Liddelow, S. A. (2019) 'Modern approaches to investigating non-neuronal aspects of Alzheimer's disease', *Faseb j,* 33(2), pp. 1528-1535.

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., Bennett, M. L., Münch, A. E., Chung, W. S., Peterson, T. C., Wilton, D. K., Frouin, A., Napier, B. A., Panicker, N., Kumar, M., Buckwalter, M. S., Rowitch, D. H.,

Dawson, V. L., Dawson, T. M., Stevens, B. and Barres, B. A. (2017) 'Neurotoxic reactive astrocytes are induced by activated microglia', *Nature,* 541(7638), pp. 481-487.

Liebsch, G., Montkowski, A., Holsboer, F. and Landgraf, R. (1998) 'Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour', *Behav Brain Res,* 94(2), pp. 301-10.

Linton, P. J. and Dorshkind, K. (2004) 'Age-related changes in lymphocyte development and function', *Nature Immunology,* 5(2), pp. 133-139.

Lister, J. P. and Barnes, C. A. (2009) 'Neurobiological Changes in the Hippocampus During Normative Aging', *Archives of Neurology,* 66(7), pp. 829-833.

Lister, R. G. (1987) 'The use of a plus-maze to measure anxiety in the mouse', *Psychopharmacology (Berl),* 92(2), pp. 180-5.

Liu, X. and Carter, A. G. (2018) 'Ventral Hippocampal Inputs Preferentially Drive Corticocortical Neurons in the Infralimbic Prefrontal Cortex', *J Neurosci,* 38(33), pp. 7351-7363.

Loerch, P. M., Lu, T., Dakin, K. A., Vann, J. M., Isaacs, A., Geula, C., Wang, J., Pan, Y., Gabuzda, D. H., Li, C., Prolla, T. A. and Yankner, B. A. (2008) 'Evolution of the aging brain transcriptome and synaptic regulation', *PLoS One,* 3(10), pp. e3329.

Long, J. Y., Chen, J. M., Liao, Y. J., Zhou, Y. J., Liang, B. Y. and Zhou, Y. (2020) 'Naringin provides neuroprotection in CCL2-induced cognition impairment by attenuating neuronal apoptosis in the hippocampus', *Behavioral and Brain Functions,* 16(1).

Lopez-Rojas, J. and Kreutz, M. R. (2016) 'Mature granule cells of the dentate gyrus-Passive bystanders or principal performers in hippocampal function?', *Neuroscience and Biobehavioral Reviews,* 64, pp. 167-174.

Lu, B., Su, Y., Das, S., Liu, J., Xia, J. and Ren, D. (2007) 'The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm', *Cell,* 129(2), pp. 371-83.

Lu, C. B., Hamilton, J. B., Powell, A. D., Toescu, E. C. and Vreugdenhil, M. (2011) 'Effect of ageing on CA3 interneuron sAHP and gamma oscillations is activity-dependent', *Neurobiology of Aging,* 32(5), pp. 956-965.

Luo, X. G., Ding, J. Q. and Chen, S. D. (2010) 'Microglia in the aging brain: relevance to neurodegeneration', *Molecular Neurodegeneration,* 5.

Lynch, M. A. (2010) 'Age-related neuroinflammatory changes negatively impact on neuronal function', *Frontiers in Aging Neuroscience,* 1, pp. 8.

Lyons, A., Lynch, A. M., Downer, E. J., Hanley, R., O'Sullivan, J. B., Smith, A. and Lynch, M. A. (2009) 'Fractalkine-induced activation of the phosphatidylinositol-3 kinase pathway attentuates microglial activation in vivo and in vitro', *Journal of Neurochemistry,* 110(5), pp. 1547-1556.

Macdonald, K. D., Fifkova, E., Jones, M. S. and Barth, D. S. (1998) 'Focal stimulation of the thalamic reticular nucleus induces focal gamma waves in cortex', *J Neurophysiol,* 79(1), pp. 474-7.

MacIver, M. B., Tanelian, D. L. and Mody, I. (1991) 'Two mechanisms for anesthetic-induced enhancement of GABAA-mediated neuronal inhibition', *Ann N Y Acad Sci,* 625, pp. 91-6.

Madar, A. D., Ewell, L. A. and Jones, M. V. (2019) 'Pattern separation of spiketrains in hippocampal neurons', *Scientific Reports,* 9.

Maguire, E. A., Frackowiak, R. S. J. and Frith, C. D. (1997) 'Recalling routes around London: Activation of the right hippocampus in taxi drivers', *Journal of Neuroscience,* 17(18), pp. 7103-7110.

Maguire, E. A., Nannery, R. and Spiers, H. J. (2006) 'Navigation around London by a taxi driver with bilateral hippocampal lesions', *Brain,* 129(Pt 11), pp. 2894-907.

Mahad, D. J. and Ransohoff, R. M. (2003) 'The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE)', *Seminars in Immunology,* 15(1), pp. 23-32.

Mahbub, S., Brubaker, A. L. and Kovacs, E. J. (2011) 'Aging of the Innate Immune System: An Update', *Curr Immunol Rev,* 7(1), pp. 104-115.

Maheswaran, S., Barjat, H., Rueckert, D., Bate, S. T., Howlett, D. R., Tilling, L., Smart, S. C., Pohlmann, A., Richardson, J. C., Hartkens, T., Hill, D. L. G., Upton, N., Hajnal, J. V. and James, M. F. (2009) 'Longitudinal regional brain volume changes quantified in normal aging and Alzheimer's APP x PS1 mice using MRI', *Brain Research,* 1270, pp. 19-32.

Mangalmurti, N. S., Xiong, Z. Y., Hulver, M., Ranganathan, M., Liu, X. H., Oriss, T., Fitzpatrick, M., Rubin, M., Triulzi, D., Choi, A. and Lee, J. S. (2009) 'Loss of red cell chemokine scavenging promotes transfusion-related lung inflammation', *Blood,* 113(5), pp. 1158-1166.

Mangold, C. A., Masser, D. R., Stanford, D. R., Bixler, G. V., Pisupati, A., Giles, C. B., Wren, J. D., Ford, M. M., Sonntag, W. E. and Freeman, W. M. (2017) 'CNS-wide Sexually Dimorphic Induction of the Major Histocompatibility Complex 1 Pathway With Aging', *J Gerontol A Biol Sci Med Sci,* 72(1), pp. 16-29.

Mansour, H., Chamberlain, C. G., Weible, M. W., 2nd, Hughes, S., Chu, Y. and Chan-Ling, T. (2008) 'Aging-related changes in astrocytes in the rat retina: imbalance between cell proliferation and cell death reduces astrocyte availability', *Aging Cell,* 7(4), pp. 526-40.

Mari, D., Mannucci, P. M., Coppola, R., Bottasso, B., Bauer, K. A. and Rosenberg, R. D. (1995) 'Hypercoagulability in the centenarians - the paradox of successful aging', *Blood,* 85(11), pp. 3144-3149.

Marrone, D. F., Satvat, E., Shaner, M. J., Worley, P. F. and Barnes, C. A. (2012) 'Attenuated long-term Arc expression in the aged fascia dentata', *Neurobiology of Aging,* 33(5), pp. 979-990.

Marsland, A. L., Gianaros, P. J., Kuan, D. C. H., Sheu, L. K., Krajina, K. and Manuck, S. B. (2015) 'Brain morphology links systemic inflammation to cognitive function in midlife adults', *Brain Behavior and Immunity,* 48, pp. 195-204.

Martin, D. S. D., Lonergan, P. E., Boland, B., Fogarty, M. P., Brady, M., Horrobin, D. F., Campbell, V. A. and Lynch, M. A. (2002) 'Apoptotic changes in the aged brain are triggered by interleukin-1 beta-induced activation of p38 and reversed by treatment with eicosapentaenoic acid', *Journal of Biological Chemistry,* 277(37), pp. 34239-34246.

Matsumura, K. and Kobayashi, S. (2004) 'Signaling the brain in inflammation: the role of endothelial cells', *Front Biosci,* 9, pp. 2819-26.

McEwen, B. S. (2007) 'Physiology and neurobiology of stress and adaptation: central role of the brain', *Physiol Rev,* 87(3), pp. 873-904.

McEwen, B. S., Conrad, C. D., Kuroda, Y., Frankfurt, M., Magarinos, A. M. and McKittrick, C. (1997) 'Prevention of stress-induced morphological and cognitive consequences', *European Neuropsychopharmacology,* 7, pp. 323-328.

McGeer, P. L., Itagaki, S., Boyes, B. E. and McGeer, E. G. (1988) 'Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains', *Neurology,* 38(8), pp. 1285-91.

McGeer, P. L. and McGeer, E. G. (2004) 'Inflammation and the degenerative diseases of aging', *Ann N Y Acad Sci,* 1035, pp. 104-16.

McHugh, S. B., Deacon, R. M. J., Rawlins, J. N. P. and Bannerman, D. M. (2004) 'Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety', *Behavioral Neuroscience,* 118(1), pp. 63-78.

McHugh, S. B., Fillenz, M., Lowry, J. P., Rawlins, J. N. and Bannerman, D. M. (2011) 'Brain tissue oxygen amperometry in behaving rats demonstrates functional dissociation of dorsal and ventral hippocampus during spatial processing and anxiety', *Eur J Neurosci,* 33(2), pp. 322-37.

McNaughton, B. L. (1980) 'Evidence for two physiologically distinct perforant pathways to the fascia dentata', *Brain Res,* 199(1), pp. 1-19.

Medzhitov, R. (2007) 'Recognition of microorganisms and activation of the immune response', *Nature,* 449(7164), pp. 819-26.

Medzhitov, R., Schneider, D. S. and Soares, M. P. (2012) 'Disease tolerance as a defense strategy', *Science,* 335(6071), pp. 936-41.

Meijer, O. C., Buurstede, J. C. and Schaaf, M. J. M. (2019) 'Corticosteroid Receptors in the Brain: Transcriptional Mechanisms for Specificity and Context-Dependent Effects', *Cell Mol Neurobiol,* 39(4), pp. 539-549.

Melik-Parsadaniantz, S. and Rostene, W. (2008) 'Chemokines and neuromodulation', *Journal of Neuroimmunology,* 198(1-2), pp. 62-68.

Merrill, D. A., Chiba, A. A. and Tuszynski, M. H. (2001) 'Conservation of neuronal number and size in the entorhinal cortex of behaviorally characterized aged rats', *J Comp Neurol,* 438(4), pp. 445-56.

Meucci, O., Fatatis, A., Simen, A. A., Bushell, T. J., Gray, P. W. and Miller, R. J. (1998) 'Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity', *Proceedings of the National Academy of Sciences of the United States of America,* 95(24), pp. 14500-14505.

Miller, A. H., Maletic, V. and Raison, C. L. (2009) 'Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression', *Biological Psychiatry,* 65(9), pp. 732-741.

Miller, L. H., Mason, S. J., Dvorak, J. A., McGinniss, M. H. and Rothman, I. K. (1975) 'Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants', *Science,* 189(4202), pp. 561-3.

Milner, T. A., Loy, R. and Amaral, D. G. (1983) 'An anatomical study of the development of the septo-hippocampal projection in the rat', *Brain Res,* 284(2-3), pp. 343-71.

Min, S. S., Quan, H. Y., Ma, J., Han, J. S., Jeon, B. H. and Seol, G. H. (2009) 'Chronic brain inflammation impairs two forms of long-term potentiation in the rat hippocampal CA1 area', *Neurosci Lett,* 456(1), pp. 20-4.

Minami, M. and Satoh, M. (2003) 'Chemokines and their receptors in the brain: Pathophysiological roles in ischemic brain injury', *Life Sciences,* 74(2-3), pp. 321-327.

Minogue, A. M., Jones, R. S., Kelly, R. J., McDonald, C. L., Connor, T. J. and Lynch, M. A. (2014) 'Age-associated dysregulation of microglial activation is coupled with enhanced blood-brain barrier permeability and pathology in APP/PS1 mice', *Neurobiology of Aging,* 35(6), pp. 1442-1452.

Minten, C., Alt, C., Gentner, M., Frei, E., Deutsch, U., Lyck, R., Schaeren-Wiemers, N., Rot, A. and Engelhardt, B. (2014) 'DARC shuttles inflammatory chemokines across the blood-brain barrier during autoimmune central nervous system inflammation', *Brain,* 137, pp. 1454-1469.

Minton, K. (2018) 'Moving on up', *Nature Reviews Immunology,* 18(1), pp. 1-1.

Mirzadegan, T., Diehl, F., Ebi, B., Bhakta, S., Polsky, I., McCarley, D., Mulkins, M., Weatherhead, G. S., Lapierre, J. M., Dankwardt, J., Morgans, D., Wilhelm, R. and Jarnagin, K. (2000) 'Identification of the binding site for a novel class of CCR2b chemokine receptor antagonists - Binding to a common chemokine receptor motif within the helical bundle', *Journal of Biological Chemistry,* 275(33), pp. 25562-25571.

Mishkin, M. (1978) 'Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus', *Nature,* 273(5660), pp. 297-8.

Mishra, P. and Narayanan, R. (2021) 'Ion-channel regulation of response decorrelation in a heterogeneous multi-scale model of the dentate gyrus', *Curr Res Neurobiol,* 2, pp. 100007.

Mitchell, S. J., Rawlins, J. N., Steward, O. and Olton, D. S. (1982) 'Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats', *J Neurosci,* 2(3), pp. 292-302.

Mizumori, S. J. Y., Lavoie, A. M. and Kalyani, A. (1996) 'Redistribution of spatial representation in the hippocampus of aged rats performing a spatial memory task', *Behavioral Neuroscience,* 110(5), pp. 1006-1016.

Moffat, S. D., Zonderman, A. B. and Resnick, S. M. (2001) 'Age differences in spatial memory in a virtual environment navigation task', *Neurobiology of Aging,* 22(5), pp. 787-796.

Moller, T. C., Hottin, J., Clerte, C., Zwier, J. M., Durroux, T., Rondard, P., Prezeau, L., Royer, C. A., Pin, J. P., Margeat, E. and Kniazeff, J. (2018) 'Oligomerization of a G protein-coupled receptor in neurons controlled by its structural dynamics', *Scientific Reports,* 8.

Monje, M. L., Toda, H. and Palmer, T. D. (2003) 'Inflammatory blockade restores adult hippocampal neurogenesis', *Science,* 302(5651), pp. 1760-1765.

Montagrin, A., Saiote, C. and Schiller, D. (2018) 'The social hippocampus', *Hippocampus,* 28(9), pp. 672-679.

Montecino-Rodriguez, E., Berent-Maoz, B. and Dorshkind, K. (2013) 'Causes, consequences, and reversal of immune system aging', *Journal of Clinical Investigation,* 123(3), pp. 958-965.

Montgomery, R. R. and Shaw, A. C. (2015) 'Paradoxical changes in innate immunity in aging: recent progress and new directions', *J Leukoc Biol,* 98(6), pp. 937-43.

Moore, J. E., Jr., Brook, B. S. and Nibbs, R. J. B. (2018) 'Chemokine Transport Dynamics and Emerging Recognition of Their Role in Immune Function', *Curr Opin Biomed Eng,* 5, pp. 90-95.

Morales, I., Guzman-Martinez, L., Cerda-Troncoso, C., Farias, G. A. and Maccioni, R. B. (2014) 'Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches', *Frontiers in Cellular Neuroscience,* 8, pp. 9.

Mormann, F., Fell, J., Axmacher, N., Weber, B., Lehnertz, K., Elger, C. E. and Fernandez, G. (2005) 'Phase/amplitude reset and theta-gamma interaction in the human medial temporal lobe during a continuous word recognition memory task', *Hippocampus,* 15(7), pp. 890-900.

Morris, R. G., Garrud, P., Rawlins, J. N. and O'Keefe, J. (1982) 'Place navigation impaired in rats with hippocampal lesions', *Nature,* 297(5868), pp. 681-3.

Morris, R. G., Schenk, F., Tweedie, F. and Jarrard, L. E. (1990) 'Ibotenate Lesions of Hippocampus and/or Subiculum: Dissociating Components of Allocentric Spatial Learning', *Eur J Neurosci,* 2(12), pp. 1016-1028.

Moser, E., Moser, M. B. and Andersen, P. (1993) 'Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions', *J Neurosci,* 13(9), pp. 3916-25.

Moser, E. I., Roudi, Y., Witter, M. P., Kentros, C., Bonhoeffer, T. and Moser, M. B. (2014) 'Grid cells and cortical representation', *Nature Reviews Neuroscience,* 15(7), pp. 466-481.

Moser, M. B. and Moser, E. I. (1998) 'Functional differentiation in the hippocampus', *Hippocampus,* 8(6), pp. 608-19.

Moser, M. B., Moser, E. I., Forrest, E., Andersen, P. and Morris, R. G. (1995) 'Spatial learning with a minislab in the dorsal hippocampus', *Proc Natl Acad Sci U S A,* 92(21), pp. 9697-701.

Mouton, P. R., Long, J. M., Lei, D. L., Howard, V., Jucker, M., Calhoun, M. E. and Ingram, D. K. (2002) 'Age and gender effects on microglia and astrocyte numbers in brains of mice', *Brain Research,* 956(1), pp. 30-35.

Mueller, S. G., Schuff, N., Yaffe, K., Madison, C., Miller, B. and Weiner, M. W. (2010) 'Hippocampal Atrophy Patterns in Mild Cognitive Impairment and Alzheimer's Disease', *Human Brain Mapping,* 31(9), pp. 1339-1347.

Mueller, S. G. and Weiner, M. W. (2009) 'Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields', *Hippocampus,* 19(6), pp. 558-64.

Muir, G. M. and Bilkey, D. K. (2001) 'Instability in the place field location of hippocampal place cells after lesions centered on the perirhinal cortex', *J Neurosci,* 21(11), pp. 4016-25.

Muller, R. U. and Kubie, J. L. (1987) 'The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells', *J Neurosci,* 7(7), pp. 1951-68.

Muller, R. U., Kubie, J. L. and Ranck, J. B., Jr. (1987) 'Spatial firing patterns of hippocampal complex-spike cells in a fixed environment', *J Neurosci,* 7(7), pp. 1935-50.

Muller, R. U., Stead, M. and Pach, J. (1996) 'The hippocampus as a cognitive graph', *J Gen Physiol,* 107(6), pp. 663-94.

Murdoch, C. and Finn, A. (2000) 'Chemokine receptors and their role in inflammation and infectious diseases', *Blood,* 95(10), pp. 3032-43.

Murty, D., Manikandan, K., Kumar, W. S., Ramesh, R. G., Purokayastha, S., Javali, M., Rao, N. P. and Ray, S. (2020) 'Gamma oscillations weaken with age in healthy elderly in human EEG', *Neuroimage,* 215, pp. 116826.

Musaeus, C. S., Nielsen, M. S., Musaeus, J. S. and Høgh, P. (2020) 'Electroencephalographic Cross-Frequency Coupling as a Sign of Disease Progression in Patients With Mild Cognitive Impairment: A Pilot Study', *Front Neurosci,* 14, pp. 790.

Naber, P. A., Caballero-Bleda, M., Jorritsma-Byham, B. and Witter, M. P. (1997) 'Parallel input to the hippocampal memory system through peri- and postrhinal cortices', *Neuroreport,* 8(11), pp. 2617-21.

Naber, P. A. and Witter, M. P. (1998) 'Subicular efferents are organized mostly as parallel projections: A double-labeling, retrograde-tracing study in the rat', *Journal of Comparative Neurology,* 393(3), pp. 284-297.

Nadeau, S. and Rivest, S. (2000) 'Role of microglial-derived tumor necrosis factor in mediating CD14 transcription and nuclear factor kappa B activity in the brain during endotoxemia', *J Neurosci,* 20(9), pp. 3456-68.

Nadon, N. L. (2004) 'Maintaining aged rodents for biogerontology research', *Lab Animal,* 33(8), pp. 36-41.

Nakanishi, H. and Wu, Z. (2009) 'Microglia-aging: Roles of microglial lysosome- and mitochondria-derived reactive oxygen species in brain aging', *Behavioural Brain Research,* 201(1), pp. 1-7.

Neves, G., Cooke, S. F. and Bliss, T. V. P. (2008) 'Synaptic plasticity, memory and the hippocampus: a neural network approach to causality', *Nature Reviews Neuroscience,* 9(1), pp. 65-75.

Newman, E. L., Gillet, S. N., Climer, J. R. and Hasselmo, M. E. (2013) 'Cholinergic blockade reduces theta-gamma phase amplitude coupling and speed modulation of theta frequency consistent with behavioral effects on encoding', *J Neurosci,* 33(50), pp. 19635-46.

Newman, M. C. and Kaszniak, A. W. (2000) 'Spatial memory and aging: Performance on a human analog of the Morris water maze', *Aging Neuropsychology and Cognition,* 7(2), pp. 86-93.

Nicaise, A. M., Willis, C. M., Crocker, S. J. and Pluchino, S. (2020) 'Stem Cells of the Aging Brain', *Frontiers in Aging Neuroscience,* 12.

Nicoletti, F., Bruno, V., Ngomba, R. T., Gradini, R. and Battaglia, G. (2015) 'Metabotropic glutamate receptors as drug targets: what's new?', *Curr Opin Pharmacol,* 20, pp. 89-94.

Nikodemova, M., Small, A. L., Kimyon, R. S. and Watters, J. J. (2016) 'Age-dependent differences in microglial responses to systemic inflammation are evident as early as middle age', *Physiological Genomics,* 48(5), pp. 336-344.

Nimmerjahn, A., Kirchhoff, F. and Helmchen, F. (2005) 'Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo', *Science,* 308(5726), pp. 1314-1318.

Nolan, M. F., Malleret, G., Dudman, J. T., Buhl, D. L., Santoro, B., Gibbs, E., Vronskaya, S., Buzsaki, G., Siegelbaum, S. A., Kandel, E. R. and Morozov, A. (2004) 'A behavioral role for dendritic integration: HCN1 channels constrain spatial inputs to distal dendrites memory and plasticity at of CA1 pyramidal neurons', *Cell,* 119(5), pp. 719-732.

Nomellini, V., Gomez, C. R. and Kovacs, E. J. (2008) 'Aging and impairment of innate immunity', *Contrib Microbiol,* 15, pp. 188-205.

Novitzky-Basso, I. and Rot, A. (2012) 'Duffy antigen receptor for chemokines and its involvement in patterning and control of inflammatory chemokines', *Frontiers in Immunology,* 3.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. and Prochiantz, A. (1984) 'Magnesium gates glutamate-activated channels in mouse central neurones', *Nature,* 307(5950), pp. 462-5.

Nusser, Z. and Mody, I. (2002) 'Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells', *Journal of Neurophysiology,* 87(5), pp. 2624-2628.

Nuñez, A., de Andrés, I. and García-Austt, E. (1991) 'Relationships of nucleus reticularis pontis oralis neuronal discharge with sensory and carbachol evoked hippocampal theta rhythm', *Exp Brain Res,* 87(2), pp. 303-8.

O'Keefe, J. and Burgess, N. (1996) 'Geometric determinants of the place fields of hippocampal neurons', *Nature,* 381(6581), pp. 425-8.

O'Keefe, J. and Dostrovsky, J. (1971) 'The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat', *Brain Res,* 34(1), pp. 171-5.

O'Keefe, J. and Recce, M. L. (1993) 'Phase relationship between hippocampal place units and the EEG theta rhythm', *Hippocampus,* 3(3), pp. 317-30.

O'Keefe, J. and Speakman, A. (1987) 'Single unit activity in the rat hippocampus during a spatial memory task', *Exp Brain Res,* 68(1), pp. 1-27.

O'Leary, O. F. and Cryan, J. F. (2014) 'A ventral view on antidepressant action: roles for adult hippocampal neurogenesis along the dorsoventral axis', *Trends Pharmacol Sci,* 35(12), pp. 675-87.

O'Mara, S. M., Commins, S., Anderson, M. and Gigg, J. (2001) 'The subiculum: a review of form, physiology and function', *Prog Neurobiol,* 64(2), pp. 129-55.

O'Reilly, R. C. and McClelland, J. L. (1994) 'Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off', *Hippocampus,* 4(6), pp. 661-82.

Odegaard, A. O., Jacobs, D. R., Sanchez, O. A., Goff, D. C., Reiner, A. P. and Gross, M. D. (2016) 'Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes', *Cardiovascular Diabetology,* 15.

Olton, D. S., Walker, J. A. and Gage, F. H. (1978) 'Hippocampal connections and spatial discrimination', *Brain Res,* 139(2), pp. 295-308.

Onyszchuk, G., Al-Hafez, B., He, Y. Y., Bilgen, M., Berman, N. E. J. and Brooks, W. M. (2007) 'A mouse model of sensorimotor controlled cortical impact: Characterization using longitudinal magnetic resonance imaging, behavioral assessments and histology', *Journal of Neuroscience Methods,* 160(2), pp. 187-196.

Ousman, S. S. and Kubes, P. (2012) 'Immune surveillance in the central nervous system', *Nat Neurosci,* 15(8), pp. 1096-101.

Ozaki, K. and Leonard, W. J. (2002) 'Cytokine and cytokine receptor pleiotropy and redundancy', *J Biol Chem,* 277(33), pp. 29355-8.

Palmer, A. L. and Ousman, S. S. (2018) 'Astrocytes and Aging', *Front Aging Neurosci,* 10, pp. 337.

Paludan, S. R., Pradeu, T., Masters, S. L. and Mogensen, T. H. (2021) 'Constitutive immune mechanisms: mediators of host defence and immune regulation', *Nature Reviews Immunology,* 21(3), pp. 137-150.

Parachikova, A., Nichol, K. E. and Cotman, C. W. (2008) 'Short-term exercise in aged Tg2576 mice alters neuroinflammation and improves cognition', *Neurobiology of Disease,* 30(1), pp. 121-129.

Pastalkova, E., Serrano, P., Pinkhasova, D., Wallace, E., Fenton, A. A. and Sacktor, T. C. (2006) 'Storage of spatial information by the maintenance mechanism of LTP', *Science,* 313(5790), pp. 1141-1144.

Patel, D. D., Koopmann, W., Imai, T., Whichard, L. P., Yoshie, O. and Krangel, M. S. (2001) 'Chemokines have diverse abilities to form solid phase gradients', *Clin Immunol,* 99(1), pp. 43-52.

Patterson, A. M., Siddall, H., Chamberlain, G., Gardner, L. and Middleton, J. (2002) 'Expression of the Duffy antigen/receptor for chemokines (DARC) by the inflamed synovial endothelium', *Journal of Pathology,* 197(1), pp. 108-116.

Pawelec, G., McElhaney, J. E., Aiello, A. E. and Derhovanessian, E. (2012) 'The impact of CMV infection on survival in older humans', *Current Opinion in Immunology,* 24(4), pp. 507-511.

Pelegri, C., Canudas, A. M., del Valle, J., Casadesus, G., Smith, M. A., Camins, A., Pallas, M. and Vilaplana, J. (2007) 'Increased permeability of blood-brain barrier on the hippocampus of a murine model of senescence', *Mechanisms of Ageing and Development,* 128(9), pp. 522-528.

Pena, R. R., Pereira-Caixeta, A. R., Moraes, M. F. D. and Pereira, G. S. (2014) 'Anisomycin administered in the olfactory bulb and dorsal hippocampus impaired social recognition memory consolidation in different time-points', *Brain Research Bulletin,* 109, pp. 151-157.

Pender, N. P. (2002) 'From conditioning to conscious recollection: Memory systems of the brain', *Trends in Cognitive Sciences,* 6(5), pp. 224-225.

Perry, V. H., Cunningham, C. and Holmes, C. (2007) 'Systemic infections and inflammation affect chronic neurodegeneration', *Nat Rev Immunol,* 7(2), pp. 161-7.

Persson, J., Pudas, S., Lind, J., Kauppi, K., Nilsson, L. G. and Nyberg, L. (2012) 'Longitudinal Structure-Function Correlates in Elderly Reveal MTL Dysfunction with Cognitive Decline', *Cerebral Cortex,* 22(10), pp. 2297-2304.

Pfurtscheller, G. and Berghold, A. (1989) 'Patterns of cortical activation during planning of voluntary movement', *Electroencephalogr Clin Neurophysiol,* 72(3), pp. 250-8.

Pfurtscheller, G. and Neuper, C. (1992) 'Simultaneous EEG 10 Hz desynchronization and 40 Hz synchronization during finger movements', *Neuroreport,* 3(12), pp. 1057-60.

Pfurtscheller, G., Neuper, C. and Kalcher, J. (1993) '40-Hz oscillations during motor behavior in man', *Neurosci Lett,* 164(1-2), pp. 179-82.

Piggins, H. D. and Guilding, C. (2011) 'The neural circadian system of mammals', *Essays Biochem,* 49(1), pp. 1-17.

Pinke, K. H., Calzavara, B., Faria, P. F., do Nascimento, M. P. P., Venturini, J. and Lara, V. S. (2013) 'Proinflammatory profile of in vitro monocytes in the ageing is affected by lymphocytes presence', *Immunity & Ageing,* 10, pp. 10.

Proell, M., Riedl, S. J., Fritz, J. H., Rojas, A. M. and Schwarzenbacher, R. (2008) 'The Nod-like receptor (NLR) family: a tale of similarities and differences', *PLoS One,* 3(4), pp. e2119.

Prolla, T. A. (2002) 'DNA microarray analysis of the aging brain', *Chemical Senses,* 27(3), pp. 299-306.

Proudfoot, A. E. I. (2002) 'Chemokine receptors: Multifaceted therapeutic targets', *Nature Reviews Immunology,* 2(2), pp. 106-115.

Pruenster, M., Mudde, L., Bombosi, P., Dimitrova, S., Zsak, M., Middleton, J., Richmond, A., Graham, G., Segerer, S., Nibbs, R. and Rot, A. (2009a) 'The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity', *Journal of Vascular Research,* 46, pp. 34-34.

Pulsatelli, L., Meliconi, R., Mazzetti, I., Dolzani, P., Meneghetti, A., Neri, S., Silvestri, T., Ravaglia, G., Forti, P., Facchini, A. and Mariani, E. (2000) 'Chemokine production by peripheral blood mononuclear cells in elderly subjects', *Mechanisms of Ageing and Development,* 121(1-3), pp. 89-100.

Pöschel, B., Draguhn, A. and Heinemann, U. (2002) 'Glutamate-induced gamma oscillations in the dentate gyrus of rat hippocampal slices', *Brain Res,* 938(1-2), pp. 22-8.

Quan, N., Stern, E. L., Whiteside, M. B. and Herkenham, M. (1999) 'Induction of pro-inflammatory cytokine mRNAs in the brain after peripheral injection of subseptic doses of lipopolysaccharide in the rat', *J Neuroimmunol,* 93(1-2), pp. 72-80.

Quast, T., Zölzer, K., Guu, D., Alvarez, L., Küsters, C., Kiermaier, E., Kaupp, U. B. and Kolanus, W. (2022) 'A Stable Chemokine Gradient Controls Directional Persistence of Migrating Dendritic Cells', *Front Cell Dev Biol,* 10, pp. 943041.

Quirk, G. J., Muller, R. U. and Kubie, J. L. (1990) 'The firing of hippocampal place cells in the dark depends on the rat's recent experience', *J Neurosci,* 10(6), pp. 2008-17.

Quiroga, R. Q., Kraskov, A., Koch, C. and Fried, I. (2009) 'Explicit Encoding of Multimodal Percepts by Single Neurons in the Human Brain', *Current Biology,* 19(15), pp. 1308-1313.

Quiroga, R. Q., Reddy, L., Kreiman, G., Koch, C. and Fried, I. (2005) 'Invariant visual representation by single neurons in the human brain', *Nature,* 435(7045), pp. 1102-1107.

Radulescu, C. I., Cerar, V., Haslehurst, P., Kopanitsa, M. and Barnes, S. J. (2021) 'The aging mouse brain: cognition, connectivity and calcium', *Cell Calcium,* 94.

Raine, C. S. (1994) 'Multiple sclerosis: immune system molecule expression in the central nervous system', *J Neuropathol Exp Neurol,* 53(4), pp. 328-37.

Raisman, G., Cowan, W. M. and Powell, T. P. S. (1965) 'Extrinsic afferent commissural and association fibres of hippocampus', *Brain,* 88, pp. 963-+.

Ransohoff, R. M. (2009) 'Chemokines and Chemokine Receptors: Standing at the Crossroads of Immunobiology and Neurobiology', *Immunity,* 31(5), pp. 711-721.

Ransohoff, R. M. and Cardona, A. E. (2010) 'The myeloid cells of the central nervous system parenchyma', *Nature,* 468(7321), pp. 253-62.

Rapp, P. R., Kansky, M. T. and Roberts, J. A. (1997) 'Impaired spatial information processing in aged monkeys with preserved recognition memory', *Neuroreport,* 8(8), pp. 1923-1928.

Raven, F., Meerlo, P., Van der Zee, E. A., Abel, T. and Havekes, R. (2019) 'A brief period of sleep deprivation causes spine loss in the dentate gyrus of mice', *Neurobiology of Learning and Memory,* 160, pp. 83-90.

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A. and Pollmächer, T. (2001) 'Cytokine-associated emotional and cognitive disturbances in humans', *Arch Gen Psychiatry,* 58(5), pp. 445-52.

Reutershan, J., Harry, B., Chang, D., Bagby, G. J. and Ley, K. (2009) 'DARC on RBC limits lung injury by balancing compartmental distribution of CXC chemokines', *European Journal of Immunology,* 39(6), pp. 1597-1607.

Ribak, C. E. and Peterson, G. M. (1991) 'Intragranular mossy fibers in rats and gerbils form synapses with the somata and proximal dendrites of basket cells in the dentate gyrus', *Hippocampus,* 1(4), pp. 355-64.

Risold, P. Y. and Swanson, L. W. (1997) 'Connections of the rat lateral septal complex', *Brain Res Brain Res Rev,* 24(2-3), pp. 115-95.

Risold, P. Y., Thompson, R. H. and Swanson, L. W. (1997) 'The structural organization of connections between hypothalamus and cerebral cortex', *Brain Res Brain Res Rev,* 24(2-3), pp. 197-254.

Ritchie, K., Carriere, I., Howett, D., Su, L., Hornberger, M., O'Brien, J. T., Ritchie, C. W. and Chan, D. (2018) 'Allocentric and Egocentric Spatial Processing in Middle-Aged Adults at High Risk of Late-Onset Alzheimer's Disease: The PREVENT Dementia Study', *Journal of Alzheimers Disease,* 65(3), pp. 885-896.

Roach, J. C., Glusman, G., Rowen, L., Kaur, A., Purcell, M. K., Smith, K. D., Hood, L. E. and Aderem, A. (2005) 'The evolution of vertebrate Toll-like receptors', *Proc Natl Acad Sci U S A,* 102(27), pp. 9577-82.

Roberts, A. C., Tomic, D. L., Parkinson, C. H., Roeling, T. A., Cutter, D. J., Robbins, T. W. and Everitt, B. J. (2007) 'Forebrain connectivity of the prefrontal cortex in the marmoset monkey (Callithrix jacchus): an anterograde and retrograde tract-tracing study', *J Comp Neurol,* 502(1), pp. 86-112.

Roberts, G. W., Woodhams, P. L., Polak, J. M. and Crow, T. J. (1984) 'Distribution of neuropeptides in the limbic system of the rat: the hippocampus', *Neuroscience,* 11(1), pp. 35-77.

Robinson, R. B. (2003) 'Hyperpolarization-activated cation currents: From molecules to physiological function', *Annual Review of Physiology,* 65, pp. 453-480.

Roger (2011) 'Heart Disease and Stroke Statistics-2011 Update: A Report From the American Heart Association (vol 123, pg e18, 2011)', *Circulation,* 123(6), pp. E240-E240.

Roh, J. S. and Sohn, D. H. (2018) 'Damage-Associated Molecular Patterns in Inflammatory Diseases', *Immune Netw,* 18(4), pp. e27.

Romanovsky, A. A. (2004) 'Signaling the brain in the early sickness syndrome: are sensory nerves involved?', *Front Biosci,* 9, pp. 494-504.

Rostene, W., Kitabgi, P. and Melik-Parsadaniantz, S. (2007) 'Opinion - Chemokines: a new class of neuromodulator?', *Nature Reviews Neuroscience,* 8(11), pp. 895-904.

Routtenberg, A. (1980) 'The hippocampus as a cognitive map - Okeefe J, Nadell L', *American Journal of Psychology,* 93(1), pp. 177-178.

Ruan, J. and Yao, Y. (2020) 'Behavioral tests in rodent models of stroke', *Brain Hemorrhages,* 1(4), pp. 171-184.

Rubin, R. D., Watson, P. D., Duff, M. C. and Cohen, N. J. (2014) 'The role of the hippocampus in flexible cognition and social behavior', *Frontiers in Human Neuroscience,* 8, pp. 15.

Ruparelia, N., Chai, J. T., Fisher, E. A. and Choudhury, R. P. (2017) 'Inflammatory processes in cardiovascular disease: a route to targeted therapies', *Nature Reviews Cardiology,* 14(3).

Ruth, R. E., Collier, T. J. and Routtenberg, A. (1982) 'Topography between the entorhinal cortex and the dentate septotemporal axis in rats: I. Medial and intermediate entorhinal projecting cells', *J Comp Neurol,* 209(1), pp. 69-78.

Sadik, C. D. and Luster, A. D. (2012) 'Lipid-cytokine-chemokine cascades orchestrate leukocyte recruitment in inflammation', *J Leukoc Biol,* 91(2), pp. 207-15.

Sagar, S. M., Sharp, F. R. and Curran, T. (1988) 'Expression of c-fos protein in brain: metabolic mapping at the cellular level', *Science,* 240(4857), pp. 1328-31.

Sahay, A. and Hen, R. (2007) 'Adult hippocampal neurogenesis in depression', *Nature Neuroscience,* 10(9), pp. 1110-1115.

Sakhi, K., Belle, M. D. C., Gossan, N., Delagrange, P. and Piggins, H. D. (2014) 'Daily variation in the electrophysiological activity of mouse medial habenula neurones', *Journal of Physiology-London,* 592(4), pp. 587-603.

Salthouse, T. A. (2009) 'When does age-related cognitive decline begin?', *Neurobiology of Aging,* 30(4), pp. 507-514.

Samuelsson, B., Dahlén, S. E., Lindgren, J. A., Rouzer, C. A. and Serhan, C. N. (1987) 'Leukotrienes and lipoxins: structures, biosynthesis, and biological effects', *Science,* 237(4819), pp. 1171-6.

Sanders, H. I. and Warrington, E. K. (1971) 'Memory for remote events in amnesic patients', *Brain,* 94(4), pp. 661-8.

Sare, R. M., Lemons, A. and Smith, C. B. (2021) 'Behavior Testing in Rodents: Highlighting Potential Confounds Affecting Variability and Reproducibility', *Brain Sciences,* 11(4).

Satvat, E., Schmidt, B., Argraves, M., Marrone, D. F. and Markus, E. J. (2011) 'Changes in Task Demands Alter the Pattern of zif268 Expression in the Dentate Gyrus', *Journal of Neuroscience,* 31(19), pp. 7163-7167.

Sawada, M., Sawada, H. and Nagatsu, T. (2008) 'Effects of aging on neuroprotective and neurotoxic properties of microglia in neurodegenerative diseases', *Neurodegener Dis,* 5(3-4), pp. 254-6.

Scharfman, H. E., Sollas, A. L., Smith, K. L., Jackson, M. B. and Goodman, J. H. (2002) 'Structural and functional asymmetry in the normal and epileptic rat dentate gyrus', *Journal of Comparative Neurology,* 454(4), pp. 424-439.

Schaum, N. and Karkanias, J. and Neff, N. F. and May, A. P. and Quake, S. R. and Wyss-Coray, T. and Darmanis, S. and Batson, J. and Botvinnik, O. and Chen, M. B. and Chen, S. and Green, F. and Jones, R. C. and Maynard, A. and Penland, L. and Pisco, A. O. and Sit, R. V. and Stanley, G. M. and Webber, J. T. and Zanini, F. and Baghel, A. S. and Bakerman, I. and Bansal, I. and Berdnik, D. and Bilen, B. and Brownfield, D. and Cain, C. and Cho, M. and Cirolia, G. and Conley, S. D. and Demers, A. and Demir, K. and Morree, A. and Divita, T. and Bois, H. and Dulgeroff, L. B. T. and Ebadi, H. and Espinoza, F. H. and Fish, M. and Gan, Q. and George, B. M. and Gillich, A. and Genetiano, G. and Gu, X. and Gulati, G. S. and Hang, Y. and Hosseinzadeh, S. and Huang, A. and Iram, T. and Isobe, T. and Ives, F. and Kao, K. S. and Karnam, G. and Kershner, A. M. and Kiss, B. M. and Kong, W. and Kumar, M. E. and Lam, J. Y. and Lee, D. P. and Lee, S. E. and Li, G. and Li, Q. and Liu, L. and Lo, A. and Lu, W. J. and Manjunath, A. and May, K. L. and May, O. L. and McKay, M. and Metzger, R. J. and Mignardi, M. and Min, D. and Nabhan, A. N. and Ng, K. M. and Noh, J. and Patkar, R. and Peng, W. C. and Puccinelli, R. and Sikandar, S. S. and Sinha, R. and Szade, K. and Tan, W. and Tato, C. and Tellez, K. and Travaglini, K. J. and Tropini, C. and Waldburger, L. and Weele, L. J. and Wosczyna, M. N. and Xiang, J. and Xue, S. and Youngyunpipatkul, J. and Zardeneta, M. E. and Zhang, F. and Zhou, L. and Castro, P. and Croote, D. and DeRisi, J. L. and Kuo, C. S. and Lehallier, B. and Nguyen, P. K. and Tan, S. Y. and Wang, B. M. and Yousef, H. and Beachy, P. A. and Chan, C. K. F. and Weinberg, K. and Wu, S. M. and Barres, B. A. and Clarke, M. F. and Huang, K. C. and Kim, S. K. and Krasnow, M. A. and Nusse, R. and Rando, T. A. and Sonnenburg, J. and Weissman, I. L. and Tabula Muris, C. (2018) 'Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris', *Nature,* 562(7727), pp. 367-+.

Scheff, S. W., Price, D. A., Schmitt, F. A. and Mufson, E. J. (2006) 'Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment', *Neurobiology of Aging,* 27(10), pp. 1372-1384.

Schmidt, B., Marrone, D. F. and Markus, E. J. (2012) 'Disambiguating the similar: The dentate gyrus and pattern separation', *Behavioural Brain Research,* 226(1), pp. 56-65.

Schneider, E. H., Fowler, S. C., Lionakis, M. S., Swamydas, M., Holmes, G., Diaz, V., Munasinghe, J., Peiper, S. C., Gao, J. L. and Murphy, P. M. (2014) 'Regulation of Motor Function and Behavior by Atypical Chemokine Receptor 1', *Behavior Genetics,* 44(5), pp. 498-515.

Schreurs, A., Sabanov, V. and Balschun, D. (2017) 'Distinct Properties of Long-Term Potentiation in the Dentate Gyrus along the Dorsoventral Axis: Influence of Age and Inhibition', *Scientific Reports,* 7.

Schuff, N., Amend, D. L., Knowlton, R., Norman, D., Fein, G. and Weiner, M. W. (1999) 'Age-related metabolite changes and volume loss in the hippocampus by magnetic resonance spectroscopy and imaging', *Neurobiology of Aging,* 20(3), pp. 279-285.

Schumann, K., Lämmermann, T., Bruckner, M., Legler, D. F., Polleux, J., Spatz, J. P., Schuler, G., Förster, R., Lutz, M. B., Sorokin, L. and Sixt, M. (2010) 'Immobilized chemokine fields and soluble chemokine gradients cooperatively shape migration patterns of dendritic cells', *Immunity,* 32(5), pp. 703-13.

Seeley, E. J., Matthay, M. A. and Wolters, P. J. (2012) 'Inflection points in sepsis biology: from local defense to systemic organ injury', *Am J Physiol Lung Cell Mol Physiol,* 303(5), pp. L355-63.

Segal, M. and Barker, J. L. (1984) 'Rat hippocampal neurons in culture: voltage-clamp analysis of inhibitory synaptic connections', *J Neurophysiol,* 52(3), pp. 469-87.

Segerer, S., Regele, H., Mack, M., Kain, R., Cartron, J. P., Colin, Y., Kerjaschki, D. and Schlondorff, D. (2000) 'The Duffy antigen receptor for chemokines is up-regulated during acute renal transplant rejection and crescentic glomerulonephritis', *Kidney International,* 58(4), pp. 1546-1556.

Seidler, S., Zimmermann, H. W., Bartneck, M., Trautwein, C. and Tacke, F. (2010) 'Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults', *Bmc Immunology,* 11, pp. 11.

Semple, B. D., Bye, N., Rancan, M., Ziebell, J. M. and Morganti-Kossmann, M. C. (2010) 'Role of CCL2 (MCP-1) in traumatic brain injury (TBI): evidence from severe TBI patients and CCL2-/- mice', *Journal of Cerebral Blood Flow and Metabolism,* 30(4), pp. 769-782.

Seress, L. and Pokorny, J. (1981) 'Structure of the granular layer of the rat dentate gyrus. A light microscopic and Golgi study', *J Anat,* 133(Pt 2), pp. 181-95.

Serhan, C. N., Maddox, J. F., Petasis, N. A., Akritopoulou-Zanze, I., Papayianni, A., Brady, H. R., Colgan, S. P. and Madara, J. L. (1995) 'Design of lipoxin A4 stable analogs that block transmigration and adhesion of human neutrophils', *Biochemistry,* 34(44), pp. 14609-15.

Serhan, C. N. and Savill, J. (2005) 'Resolution of inflammation: the beginning programs the end', *Nat Immunol,* 6(12), pp. 1191-7.

Sesso, H. D., Jiménez, M. C., Wang, L., Ridker, P. M., Buring, J. E. and Gaziano, J. M. (2015) 'Plasma Inflammatory Markers and the Risk of Developing Hypertension in Men', *J Am Heart Assoc,* 4(9), pp. e001802.

Sharma, S., Yang, B., Strong, R., Xi, X., Brenneman, M., Grotta, J. C., Aronowski, J. and Savitz, S. I. (2010) 'Bone marrow mononuclear cells protect neurons and modulate microglia in cell culture models of ischemic stroke', *J Neurosci Res,* 88(13), pp. 2869-76.

Sharshar, T., Annane, D., de la Grandmaison, G. L., Brouland, J. P., Hopkinson, N. S. and Françoise, G. (2004) 'The neuropathology of septic shock', *Brain Pathol,* 14(1), pp. 21-33.

Shaw, A. C., Goldstein, D. R. and Montgomery, R. R. (2013) 'Age-dependent dysregulation of innate immunity', *Nature Reviews Immunology,* 13(12), pp. 875-887.

Shaw, J. M. and Nunnari, J. (2002) 'Mitochondrial dynamics and division in budding yeast', *Trends in Cell Biology,* 12(4), pp. 178-184.

Shen, J. M., Barnes, C. A., McNaughton, B. L., Skaggs, W. E. and Weaver, K. L. (1997) 'The effect of aging on experience-dependent plasticity of hippocampal place cells', *Journal of Neuroscience,* 17(17), pp. 6769-6782.

Shoji, H., Takao, K., Hattori, S. and Miyakawa, T. (2016) 'Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age', *Molecular Brain,* 9.

Siarey, R. J., Andreasen, M. and Lambert, J. D. (1995) 'Serotoninergic modulation of excitability in area CA1 of the in vitro rat hippocampus', *Neurosci Lett,* 199(3), pp. 211-4.

Simonyi, A., Miller, L. A. and Sun, G. Y. (2000) 'Region-specific decline in the expression of metabotropic glutamate receptor 7 mRNA in rat brain during aging', *Molecular Brain Research,* 82(1-2), pp. 101-106.

Simpson, J. E., Newcombe, J., Cuzner, M. L. and Woodroofe, M. N. (1998) 'Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions', *Journal of Neuroimmunology,* 84(2), pp. 238-249.

Singer, W. (1993) 'Synchronization of cortical activity and its putative role in information processing and learning', *Annu Rev Physiol,* 55, pp. 349-74.

Singh-Manoux, A., Dugravot, A., Brunner, E., Kumari, M., Shipley, M., Elbaz, A. and Kivimaki, M. (2014) 'Interleukin-6 and C-reactive protein as predictors of cognitive decline in late midlife', *Neurology,* 83(6), pp. 486-93.

Sinha, M. and Narayanan, R. (2015) 'HCN channels enhance spike phase coherence and regulate the phase of spikes and LFPs in the theta-frequency range', *Proceedings of the National Academy of Sciences of the United States of America,* 112(17), pp. E2207-E2216.

Sirota, A., Montgomery, S., Fujisawa, S., Isomura, Y., Zugaro, M. and Buzsáki, G. (2008) 'Entrainment of neocortical neurons and gamma oscillations by the hippocampal theta rhythm', *Neuron,* 60(4), pp. 683-97.

Sliwa, J., Planté, A., Duhamel, J. R. and Wirth, S. (2016) 'Independent Neuronal Representation of Facial and Vocal Identity in the Monkey Hippocampus and Inferotemporal Cortex', *Cereb Cortex,* 26(3), pp. 950-966.

Smith, J. A., Das, A., Ray, S. K. and Banik, N. L. (2012) 'Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases', *Brain Research Bulletin,* 87(1), pp. 10-20.

Snyder, J. S., Ferrante, S. C. and Cameron, H. A. (2012) 'Late maturation of adult-born neurons in the temporal dentate gyrus', *PLoS One,* 7(11), pp. e48757.

Snyder, J. S., Radik, R., Wojtowicz, J. M. and Cameron, H. A. (2009) 'Anatomical Gradients of Adult Neurogenesis and Activity: Young Neurons in the Ventral Dentate Gyrus Are Activated by Water Maze Training', *Hippocampus,* 19(4), pp. 360-370.

Sofroniew, M. V. and Vinters, H. V. (2010) 'Astrocytes: biology and pathology', *Acta Neuropathol,* 119(1), pp. 7-35.

Sokol, C. L. and Luster, A. D. (2015) 'The chemokine system in innate immunity', *Cold Spring Harb Perspect Biol,* 7(5).

Sokolova, A., Hill, M. D., Rahimi, F., Warden, L. A., Halliday, G. M. and Shepherd, C. E. (2009a) 'Monocyte Chemoattractant Protein-1 Plays a Dominant Role in the Chronic Inflammation Observed in Alzheimer's Disease', *Brain Pathology,* 19(3), pp. 392-398.

Solano, R. M., Casarejos, M. J., Menéndez-Cuervo, J., Rodriguez-Navarro, J. A., García de Yébenes, J. and Mena, M. A. (2008) 'Glial dysfunction in parkin null mice: effects of aging', *J Neurosci,* 28(3), pp. 598-611.

Song, L. and Pachter, J. S. (2004) 'Monocyte chemoattractant protein-1 alters expression of tight junction-associated proteins in brain microvascular endothelial cells', *Microvascular Research,* 67(1), pp. 78-89.

Sorra, K. E. and Harris, K. M. (2000) 'Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines', *Hippocampus,* 10(5), pp. 501-11.

Sousa, N., Cerqueira, J. J. and Almeida, O. F. X. (2008) 'Corticosteroid receptors and neuroplasticity', *Brain Research Reviews,* 57(2), pp. 561-570.

Sparkman, N. L., Buchanan, J. B., Heyen, J. R., Chen, J., Beverly, J. L. and Johnson, R. W. (2006) 'Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers', *J Neurosci,* 26(42), pp. 10709-16.

Spreng, R. N., Stevens, W. D., Chamberlain, J. P., Gilmore, A. W. and Schacter, D. L. (2010) 'Default network activity, coupled with the frontoparietal control network, supports goal-directed cognition', *Neuroimage,* 53(1), pp. 303-317.

Stafstrom, C. E., Schwindt, P. C. and Crill, W. E. (1982) 'Negative slope conductance due to a persistent subthreshold sodium current in cat neocortical neurons in vitro', *Brain Res,* 236(1), pp. 221-6.

Stamatovic, S. M., Keep, R. F., Kunkel, S. L. and Andjelkovic, A. V. (2003) 'Potential role of MCP-1 in endothelial cell tight junction 'opening': signaling via Rho and Rho kinase', *Journal of Cell Science,* 116(22), pp. 4615-4628.

Stamatovic, S. M., Shakui, P., Keep, R. F., Moore, B. B., Kunkel, S. L., Van Rooijen, N. and Andjelkovic, A. V. (2005) 'Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability', *Journal of Cerebral Blood Flow and Metabolism,* 25(5), pp. 593-606.

Stankovic, A., Slavic, V., Stamenkovic, B., Kamenov, B., Bojanovic, M. and Mitrovic, D. R. (2009) 'Serum and synovial fluid concentrations of CCL2 (MCP-1) chemokine in patients suffering rheumatoid arthritis and osteoarthritis reflect disease activity', *Bratislava Medical Journal-Bratislavske Lekarske Listy,* 110(10), pp. 641-646.

Stanley, E. M., Fadel, J. R. and Mott, D. D. (2012) 'Interneuron loss reduces dendritic inhibition and GABA release in hippocampus of aged rats', *Neurobiology of Aging,* 33(2).

Stark, S. M., Yassa, M. A. and Stark, C. E. L. (2010) 'Individual differences in spatial pattern separation performance associated with healthy aging in humans', *Learning & Memory,* 17(6), pp. 284-288.

Steen, A., Larsen, O., Thiele, S. and Rosenkilde, M. M. (2014) 'Biased and g protein-independent signaling of chemokine receptors', *Front Immunol,* 5, pp. 277.

Stella, F., Cerasti, E., Si, B. L., Jezek, K. and Treves, A. (2012) 'Self-organization of multiple spatial and context memories in the hippocampus', *Neuroscience and Biobehavioral Reviews,* 36(7), pp. 1609-1625.

Stern, C. E., Corkin, S., Gonzalez, R. G., Guimaraes, A. R., Baker, J. R., Jennings, P. J., Carr, C. A., Sugiura, R. M., Vedantham, V. and Rosen, B. R. (1996) 'The hippocampal formation participates in novel picture encoding: Evidence from functional magnetic resonance imaging', *Proceedings of the National Academy of Sciences of the United States of America,* 93(16), pp. 8660-8665.

Steward, O. (1976) 'Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat', *J Comp Neurol,* 167(3), pp. 285-314.

Steward, O. and Scoville, S. A. (1976) 'Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat', *J Comp Neurol,* 169(3), pp. 347-70.

Stichel, C. C. and Luebbert, H. (2007) 'Inflammatory processes in the aging mouse brain: Participation of dendritic cells and T-cells', *Neurobiology of Aging,* 28(10), pp. 1507-1521.

Strange, B. A., Witter, M. P., Lein, E. S. and Moser, E. I. (2014) 'Functional organization of the hippocampal longitudinal axis', *Nature Reviews Neuroscience,* 15(10), pp. 655-669.

Streit, W. J., Mrak, R. E. and Griffin, W. S. T. (2004) 'Microglia and neuroinflammation: a pathological perspective', *Journal of Neuroinflammation,* 1.

Streit, W. J., Sammons, N. W., Kuhns, A. J. and Sparks, D. L. (2004) 'Dystrophic microglia in the aging human brain', *Glia,* 45(2), pp. 208-12.

Strell, C. and Entschladen, F. (2008) 'Extravasation of leukocytes in comparison to tumor cells', *Cell Commun Signal,* 6, pp. 10.

Stuart, G. (1999) 'Voltage-activated sodium channels amplify inhibition in neocortical pyramidal neurons', *Nat Neurosci,* 2(2), pp. 144-50.

Stuart, G. and Sakmann, B. (1995) 'Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons', *Neuron,* 15(5), pp. 1065-76.

Stumpf, C., Petsche, H. and Gogolak, G. (1962) 'The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. II. The differential influence of drugs upon both the septal cell firing pattern and the hippocampus theta activity', *Electroencephalogr Clin Neurophysiol,* 14, pp. 212-9.

Sturman, O., Germain, P. L. and Bohacek, J. (2018) 'Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test', *Stress,* 21(5), pp. 443-452.

Su, H., Alroy, G., Kirson, E. D. and Yaari, Y. (2001) 'Extracellular calcium modulates persistent sodium current-dependent burst-firing in hippocampal pyramidal neurons', *J Neurosci,* 21(12), pp. 4173-82.

Sue, W. and Griffin, T. (2006) 'Inflammation and neurodegenerative diseases', *American Journal of Clinical Nutrition,* 83(2), pp. 470S-474S.

Sun, J. H., Yang, B., Donnelly, D. F., Ma, C. and LaMotte, R. H. (2006) 'MCP-1 enhances excitability of nociceptive neurons in chronically compressed dorsal root ganglia', *Journal of Neurophysiology,* 96(5), pp. 2189-2199.

Sutherland, R. J., Whishaw, I. Q. and Kolb, B. (1983) 'A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat', *Behav Brain Res,* 7(2), pp. 133-53.

Swanson, L. W. and Cowan, W. M. (1977) 'An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat', *J Comp Neurol,* 172(1), pp. 49-84.

Székely, M., Balaskó, M., Kulchitsky, V. A., Simons, C. T., Ivanov, A. I. and Romanovsky, A. A. (2000) 'Multiple neural mechanisms of fever', *Auton Neurosci,* 85(1-3), pp. 78-82.

Takeda, A., Baffi, J. Z., Kleinman, M. E., Cho, W. G., Nozaki, M., Yamada, K., Kaneko, H., Albuquerque, R. J., Dridi, S., Saito, K., Raisler, B. J., Budd, S. J., Geisen, P., Munitz, A., Ambati, B. K., Green, M. G., Ishibashi, T., Wright, J. D., Humbles, A. A., Gerard, C. J., Ogura, Y., Pan, Y., Smith, J. R., Grisanti, S., Hartnett, M. E., Rothenberg, M. E. and Ambati, J. (2009) 'CCR3 is a target for age-related macular degeneration diagnosis and therapy', *Nature,* 460(7252), pp. 225-30.

Tamagnini, F., Scullion, S., Brown, J. T. and Randall, A. D. (2014) 'Low concentrations of the solvent dimethyl sulphoxide alter intrinsic excitability properties of cortical and hippocampal pyramidal cells', *PLoS One,* 9(3), pp. e92557.

Tamamaki, N. (1997) 'Organization of the entorhinal projection to the rat dentate gyrus revealed by Dil anterograde labeling', *Experimental Brain Research,* 116(2), pp. 250-258.

Tamamaki, N. and Nojyo, Y. (1993) 'Project of the entorhinal layer-II neurons in the rat as revealed by intracellular pressure-injection of neurobiotin', *Hippocampus,* 3(4), pp. 471-480.

Taube, J. S. (2007) 'The head direction signal: origins and sensory-motor integration', *Annu Rev Neurosci,* 30, pp. 181-207.

Taube, J. S., Muller, R. U. and Ranck, J. B., Jr. (1990) 'Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis', *J Neurosci,* 10(2), pp. 420-35.

Tchessalova, D., Posillico, C. K. and Tronson, N. C. (2018) 'Neuroimmune Activation Drives Multiple Brain States', *Front Syst Neurosci,* 12, pp. 39.

Teschemacher, A., Zeise, M. L., Holsboer, F. and Zieglgänsberger, W. (1995) 'The neuroactive steroid 5 alpha-tetrahydrodeoxycorticosterone increases GABAergic postsynaptic inhibition in rat neocortical neurons in vitro', *J Neuroendocrinol,* 7(3), pp. 233-40.

Thacker, M. A., Clark, A. K., Bishop, T., Grist, J., Yip, P. K., Moon, L. D. F., Thompson, S. W. N., Marchand, F. and McMahon, S. B. (2009) 'CCL2 is a key mediator of microglia activation in neuropathic pain states', *European Journal of Pain,* 13(3), pp. 263-272.

Thames, A. D., Briones, M. S., Magpantay, L. I., Martinez-Maza, O., Singer, E. J., Hinkin, C. H., Morgello, S., Gelman, B. B., Moore, D. J., Heizerling, K. and Levine, A. J. (2015) 'The role of chemokine C-C motif ligand 2 genotype and cerebrospinal fluid chemokine C-C motif ligand 2 in neurocognition among HIV-infected patients', *Aids,* 29(12), pp. 1483-1491.

Thomson, C. A., McColl, A., Graham, G. J. and Cavanagh, J. (2020) 'Sustained exposure to systemic endotoxin triggers chemokine induction in the brain followed by a rapid influx of leukocytes', *Journal of Neuroinflammation,* 17(1).

Tian, D. S., Peng, J. Y., Murugan, M., Feng, L. J., Liu, J. L., Eyo, U. B., Zhou, L. J., Mogilevsky, R., Wang, W. and Wu, L. J. (2017) 'Chemokine CCL2-CCR2 Signaling Induces Neuronal Cell Death via STAT3 Activation and IL-1 beta Production after Status Epilepticus', *Journal of Neuroscience,* 37(33), pp. 7878-7892.

Tischmeyer, W. and Grimm, R. (1999) 'Activation of immediate early genes and memory formation', *Cellular and Molecular Life Sciences,* 55(4), pp. 564-574.

Toner, C. K., Pirogovsky, E., Kirwan, C. B. and Gilbert, P. E. (2009) 'Visual object pattern separation deficits in nondemented older adults', *Learn Mem,* 16(5), pp. 338-42.

Towers, S. K., LeBeau, F. E., Gloveli, T., Traub, R. D., Whittington, M. A. and Buhl, E. H. (2002) 'Fast network oscillations in the rat dentate gyrus in vitro', *J Neurophysiol,* 87(2), pp. 1165-8.

Toyoda, H., Li, X. Y., Wu, L. J., Zhao, M. G., Descalzi, G., Chen, T., Koga, K. and Zhuo, M. (2011) 'Interplay of Amygdala and Cingulate Plasticity in Emotional Fear', *Neural Plasticity,* 2011, pp. 9.

Tran, T., Bridi, M., Koh, M. T., Gallagher, M. and Kirkwood, A. (2019) 'Reduced cognitive performance in aged rats correlates with increased excitation/inhibition ratio

in the dentate gyrus in response to lateral entorhinal input', *Neurobiol Aging,* 82, pp. 120-127.

Tran, T., Gallagher, M. and Kirkwood, A. (2018) 'Enhanced postsynaptic inhibitory strength in hippocampal principal cells in high-performing aged rats', *Neurobiology of Aging,* 70, pp. 92-101.

Traub, R. D., Cunningham, M. O., Gloveli, T., LeBeau, F. E., Bibbig, A., Buhl, E. H. and Whittington, M. A. (2003) 'GABA-enhanced collective behavior in neuronal axons underlies persistent gamma-frequency oscillations', *Proc Natl Acad Sci U S A,* 100(19), pp. 11047-52.

Traub, R. D., Whittington, M. A., Buhl, E. H., Jefferys, J. G. and Faulkner, H. J. (1999) 'On the mechanism of the gamma --> beta frequency shift in neuronal oscillations induced in rat hippocampal slices by tetanic stimulation', *J Neurosci,* 19(3), pp. 1088-105.

Traub, R. D., Whittington, M. A., Colling, S. B., Buzsáki, G. and Jefferys, J. G. (1996) 'Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo', *J Physiol,* 493 (Pt 2)(Pt 2), pp. 471-84.

Tremblay, M. E., Lowery, R. L. and Majewska, A. K. (2010) 'Microglial Interactions with Synapses Are Modulated by Visual Experience', *Plos Biology,* 8(11).

Tremblay, M. E., Zettel, M. L., Ison, J. R., Allen, P. D. and Majewska, A. K. (2012) 'Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices', *Glia,* 60(4), pp. 541-558.

Trinkler, I., King, J. A., Doeller, C. F., Rugg, M. D. and Burgess, N. (2009) 'Neural Bases of Autobiographical Support for Episodic Recollection of Faces', *Hippocampus,* 19(8), pp. 718-730.

Tsao, A., Moser, M. B. and Moser, E. I. (2013) 'Traces of Experience in the Lateral Entorhinal Cortex', *Current Biology,* 23(5), pp. 399-405.

Tulving, E. (1984) 'Precis of tulving elements of episodic memory', *Behavioral and Brain Sciences,* 7(2), pp. 223-238.

Tulving, E. (1985) 'How many memory-systems are there', *American Psychologist,* 40(4), pp. 385-398.

Turini, M. E. and DuBois, R. N. (2002) 'Cyclooxygenase-2: a therapeutic target', *Annu Rev Med,* 53, pp. 35-57.

Uekita, T. and Okanoya, K. (2011) 'Hippocampus lesions induced deficits in social and spatial recognition in Octodon degus', *Behav Brain Res,* 219(2), pp. 302-9.

Uhlhaas, P. J., Pipa, G., Neuenschwander, S., Wibral, M. and Singer, W. (2011) 'A new look at gamma? High- (>60 Hz) γ-band activity in cortical networks: function, mechanisms and impairment', *Prog Biophys Mol Biol,* 105(1-2), pp. 14-28.

Ukai, M., Shinkai, N. and Kameyama, T. (1996) 'Neurokinin A and senktide attenuate scopolamine-induced impairment of spontaneous alternation performance in mice', *Nihon Shinkei Seishin Yakurigaku Zasshi,* 16(3), pp. 97-101.

Van der Kloot, W. (1991) 'The regulation of quantal size', *Prog Neurobiol,* 36(2), pp. 93-130.

van der Meer, P., Ulrich, A. M., Gonźalez-Scarano, F. and Lavi, E. (2000) 'Immunohistochemical analysis of CCR2, CCR3, CCR5, and CXCR4 in the human brain: potential mechanisms for HIV dementia', *Exp Mol Pathol,* 69(3), pp. 192-201.

van der Poll, T., van de Veerdonk, F. L., Scicluna, B. P. and Netea, M. G. (2017) 'The immunopathology of sepsis and potential therapeutic targets', *Nature Reviews Immunology,* 17(7), pp. 407-420.

Van Groen, T. and Wyss, J. M. (2003) 'Connections of the retrosplenial granular b cortex in the rat', *J Comp Neurol,* 463(3), pp. 249-63.

Van Hoesen, G. W., Hyman, B. T. and Damasio, A. R. (1991) 'Entorhinal cortex pathology in Alzheimer's disease', *Hippocampus,* 1(1), pp. 1-8.

Van Hoeymissen, E., Philippaert, K., Vennekens, R., Vriens, J. and Held, K. (2020) 'Horizontal Hippocampal Slices of the Mouse Brain', *Jove-Journal of Visualized Experiments,* (163).

van Strien, N. M., Cappaert, N. L. M. and Witter, M. P. (2009) 'The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network', *Nature Reviews Neuroscience,* 10(4), pp. 272-282.

Vanhooren, V. and Libert, C. (2013) 'The mouse as a model organism in aging research: usefulness, pitfalls and possibilities', *Ageing Res Rev,* 12(1), pp. 8-21.

Vann, S. D., Brown, M. W., Erichsen, J. T. and Aggleton, J. P. (2000) 'Fos imaging reveals differential patterns of hippocampal and parahippocampal subfield activation in rats in response to different spatial memory tests', *Journal of Neuroscience,* 20(7), pp. 2711-2718.

Vela, J., Gutierrez, A., Vitorica, J. and Ruano, D. (2003) 'Rat hippocampal GABAergic molecular markers are differentially affected by ageing', *Journal of Neurochemistry,* 85(2), pp. 368-377.

Verney, C., Baulac, M., Berger, B., Alvarez, C., Vigny, A. and Helle, K. B. (1985) 'Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat', *Neuroscience,* 14(4), pp. 1039-52.

Vezzani, A. and Granata, T. (2005) 'Brain inflammation in epilepsy: experimental and clinical evidence', *Epilepsia,* 46(11), pp. 1724-43.

Villeda, S. A., Luo, J., Mosher, K. I., Zou, B. D., Britschgi, M., Bieri, G., Stan, T. M., Fainberg, N., Ding, Z. Q., Eggel, A., Lucin, K. M., Czirr, E., Park, J. S., Couillard-Despres, S., Aigner, L., Li, G., Peskind, E. R., Kaye, J. A., Quinn, J. F., Galasko, D. R., Xie, X. M. S., Rando, T. A. and Wyss-Coray, T. (2011) 'The ageing systemic milieu negatively regulates neurogenesis and cognitive function', *Nature,* 477(7362), pp. 90-U157.

Viskontas, I. V., Quiroga, R. Q. and Fried, I. (2009) 'Human medial temporal lobe neurons respond preferentially to personally relevant images', *Proceedings of the National Academy of Sciences of the United States of America,* 106(50), pp. 21329-21334.

Vogt, B. A. and Miller, M. W. (1983) 'Cortical connections between rat cingulate cortex and visual, motor, and postsubicular cortices', *J Comp Neurol,* 216(2), pp. 192-210.

von Bohlen und Halbach, O. and Unsicker, K. (2002) 'Morphological alterations in the amygdala and hippocampus of mice during ageing', *European Journal of Neuroscience,* 16(12), pp. 2434-2440.

Vreugdenhil, M. and Toescu, E. C. (2005) 'Age-dependent reduction of gamma oscillations in the mouse hippocampus in vitro', *Neuroscience,* 132(4), pp. 1151-7.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S. and Nabekura, J. (2009) 'Resting Microglia Directly Monitor the Functional State of Synapses In Vivo and Determine the Fate of Ischemic Terminals', *Journal of Neuroscience,* 29(13), pp. 3974-3980.

Wan, Q., Xiong, Z. G., Man, H. Y., Ackerley, C. A., Braunton, J., Lu, W. Y., Becker, L. E., MacDonald, J. F. and Wang, Y. T. (1997) 'Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin', *Nature,* 388(6643), pp. 686-90.

Wang, R., Tang, Y., Feng, B., Ye, C., Fang, L., Zhang, L. and Li, L. (2007) 'Changes in hippocampal synapses and learning-memory abilities in age-increasing rats and effects of tetrahydroxystilbene glucoside in aged rats', *Neuroscience,* 149(4), pp. 739-746.

Wang, X., Xu, Y., Wang, F., Tang, L., Liu, Z., Li, H. and Liu, S. (2006) 'Aging-related changes of microglia and astrocytes in hypothalamus after intraperitoneal injection of hypertonic saline in rats', *J Huazhong Univ Sci Technolog Med Sci,* 26(2), pp. 231-4.

Wang, X. J. and Buzsáki, G. (1996) 'Gamma oscillation by synaptic inhibition in a hippocampal interneuronal network model', *J Neurosci,* 16(20), pp. 6402-13.

Wasserman, J. K., Yang, H. and Schlichter, L. C. (2008) 'Glial responses, neuron death and lesion resolution after intracerebral hemorrhage in young vs. aged rats', *Eur J Neurosci,* 28(7), pp. 1316-28.

Weaver, J. D., Huang, M. H., Albert, M., Harris, T., Rowe, J. W. and Seeman, T. E. (2002) 'Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging', *Neurology,* 59(3), pp. 371-8.

Wells, T. N. C., Power, C. A., Shaw, J. P. and Proudfoot, A. E. I. (2006) 'Chemokine blockers - therapeutics in the making?', *Trends in Pharmacological Sciences,* 27(1), pp. 41-47.

Wersching, H., Duning, T., Lohmann, H., Mohammadi, S., Stehling, C., Fobker, M., Conty, M., Minnerup, J., Ringelstein, E. B., Berger, K., Deppe, M. and Knecht, S. (2010) 'Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function', *Neurology,* 74(13), pp. 1022-9.

West, M. J. (1993) 'Regionally specific loss of neurons in the aging human hippocampus', *Neurobiol Aging,* 14(4), pp. 287-93.

West, M. J., Coleman, P. D., Flood, D. G. and Troncoso, J. C. (1994) 'Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease', *Lancet,* 344(8925), pp. 769-72.

Westin, K., Buchhave, P., Nielsen, H., Minthon, L., Janciauskiene, S. and Hansson, O. (2012) 'CCL2 is associated with a faster rate of cognitive decline during early stages of Alzheimer's disease', *PLoS One,* 7(1), pp. e30525.

Weyand, C. M. and Goronzy, J. J. (2016) 'Aging of the Immune System Mechanisms and Therapeutic Targets', *Annals of the American Thoracic Society,* 13, pp. S422-S428.

Wheeler, M. A. and Quintana, F. J. (2019) 'Regulation of Astrocyte Functions in Multiple Sclerosis', *Cold Spring Harb Perspect Med,* 9(1).

Whishaw, I. Q., Cassel, J. C. and Jarrad, L. E. (1995) 'Rats with fimbria-fornix lesions display a place response in a swimming pool: a dissociation between getting there and knowing where', *J Neurosci,* 15(8), pp. 5779-88.

White, F. A., Sun, J. H., Waters, S. M., Ma, C., Ren, D. J., Ripsch, M., Steflik, J., Cortright, D. N., LaMotte, R. H. and Miller, R. J. (2005) 'Excitatory monocyte chemoattractant protein-1 signaling is up-regulated in sensory neurons after chronic compression of the dorsal root ganglion', *Proceedings of the National Academy of Sciences of the United States of America,* 102(39), pp. 14092-14097.

White, J. A., Chow, C. C., Ritt, J., Soto-Treviño, C. and Kopell, N. (1998) 'Synchronization and oscillatory dynamics in heterogeneous, mutually inhibited neurons', *J Comput Neurosci,* 5(1), pp. 5-16.

Whiteside, T. L. (1994) 'Cytokines and cytokine measurements in a clinical laboratory', *Clin Diagn Lab Immunol,* 1(3), pp. 257-60.

Whittington, M. A., Cunningham, M. O., LeBeau, F. E., Racca, C. and Traub, R. D. (2011) 'Multiple origins of the cortical γ rhythm', *Dev Neurobiol,* 71(1), pp. 92-106.

Whittington, M. A., Stanford, I. M., Colling, S. B., Jefferys, J. G. and Traub, R. D. (1997) 'Spatiotemporal patterns of gamma frequency oscillations tetanically induced in the rat hippocampal slice', *J Physiol,* 502 (Pt 3)(Pt 3), pp. 591-607.

Whittington, M. A. and Traub, R. D. (2003) 'Interneuron diversity series: inhibitory interneurons and network oscillations in vitro', *Trends Neurosci,* 26(12), pp. 676-82.

Whittington, M. A., Traub, R. D. and Jefferys, J. G. (1995) 'Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation', *Nature,* 373(6515), pp. 612-5.

Whittington, M. A., Traub, R. D., Kopell, N., Ermentrout, B. and Buhl, E. H. (2000) 'Inhibition-based rhythms: experimental and mathematical observations on network dynamics', *Int J Psychophysiol,* 38(3), pp. 315-36.

Wiggs, C. L., Weisberg, J. and Martin, A. (1999) 'Neural correlates of semantic and episodic memory retrieval', *Neuropsychologia,* 37(1), pp. 103-118.

Williams, M. E., Graves, L. V., DeJesus, S. Y., Holden, H. M., DeFord, N. E. and Gilbert, P. E. (2019) 'Spatial memory ability during middle age may depend on level of spatial similarity', *Learning & Memory,* 26(1), pp. 20-23.

Wilson, M. A. and McNaughton, B. L. (1993) 'Dynamics of the hippocampal ensemble code for space', *Science,* 261(5124), pp. 1055-8.

Witter, M. P. (2007) 'Intrinsic and extrinsic wiring of CA3: Indications for connectional heterogeneity', *Learning & Memory,* 14(11), pp. 705-713.

Witter, M. P. and Groenewegen, H. J. (1984) 'Laminar origin and septotemporal distribution of entorhinal and perirhinal projections to the hippocampus in the cat', *J Comp Neurol,* 224(3), pp. 371-85.

Witter, M. P., Groenewegen, H. J., Lopes da Silva, F. H. and Lohman, A. H. (1989) 'Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region', *Prog Neurobiol,* 33(3), pp. 161-253.

Witter, M. P., Wouterlood, F. G., Naber, P. A. and Van Haeften, T. (2000) 'Anatomical organization of the parahippocampal-hippocampal network', *Ann N Y Acad Sci,* 911, pp. 1-24.

Wolf, Y., Yona, S., Kim, K. W. and Jung, S. (2013) 'Microglia, seen from the CX3CR1 angle', *Front Cell Neurosci,* 7, pp. 26.

Wong, M. L., Bongiorno, P. B., Rettori, V., McCann, S. M. and Licinio, J. (1997) 'Interleukin (IL) 1beta, IL-1 receptor antagonist, IL-10, and IL-13 gene expression in the central nervous system and anterior pituitary during systemic inflammation: pathophysiological implications', *Proc Natl Acad Sci U S A,* 94(1), pp. 227-32.

Wong, W. T. (2013) 'Microglial aging in the healthy CNS: phenotypes, drivers, and rejuvenation', *Frontiers in Cellular Neuroscience,* 7, pp. 13.

Woodson, W., Nitecka, L. and Benari, Y. (1989) 'Organization of the GABAergic system in the rat hippocampal-formation - a quantitative immunocytochemical study', *Journal of Comparative Neurology,* 280(2), pp. 254-271.

Wu, D., Huang, C. K. and Jiang, H. (2000) 'Roles of phospholipid signaling in chemoattractant-induced responses', *J Cell Sci,* 113 (Pt 17), pp. 2935-40.

Wu, W., Brickman, A. M., Luchsinger, J., Ferrazzano, P., Pichiule, P., Yoshita, M., Brown, T., DeCarli, C., Barnes, C. A., Mayeux, R., Vannucci, S. J. and Small, S. A. (2008) 'The Brain in the Age of Old: The Hippocampal Formation Is Targeted Differentially by Diseases of Late Life', *Annals of Neurology,* 64(6), pp. 698-706.

Wu, Y., Zhang, A. Q. and Yew, D. T. (2005) 'Age related changes of various markers of astrocytes in senescence-accelerated mice hippocampus', *Neurochem Int,* 46(7), pp. 565-74.

Wyss-Coray, T. (2016) 'Ageing, neurodegeneration and brain rejuvenation', *Nature,* 539(7628), pp. 180-186.

Xu, J. W., Dong, H. Q., Qian, Q. Q., Zhang, X., Wang, Y. W., Jin, W. J. and Qian, Y. N. (2017) 'Astrocyte-derived CCL2 participates in surgery-induced cognitive dysfunction and neuroinflammation via evoking microglia activation', *Behavioural Brain Research,* 332, pp. 145-153.

Yadav, D. and Sarvetnick, N. (2003) 'Cytokines and autoimmunity: redundancy defines their complex nature', *Current Opinion in Immunology,* 15(6), pp. 697-703.

Yamagata, K., Matsumura, K., Inoue, W., Shiraki, T., Suzuki, K., Yasuda, S., Sugiura, H., Cao, C., Watanabe, Y. and Kobayashi, S. (2001) 'Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever', *J Neurosci,* 21(8), pp. 2669-77.

Yamamoto, J., Suh, J., Takeuchi, D. and Tonegawa, S. (2014) 'Successful execution of working memory linked to synchronized high-frequency gamma oscillations', *Cell,* 157(4), pp. 845-57.

Yassa, M. A., Lacy, J. W., Stark, S. M., Albert, M. S., Gallagher, M. and Stark, C. E. (2011) 'Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults', *Hippocampus,* 21(9), pp. 968-79.

Yassa, M. A., Muftuler, L. T. and Stark, C. E. L. (2010) 'Ultrahigh-resolution microstructural diffusion tensor imaging reveals perforant path degradation in aged humans in vivo', *Proceedings of the National Academy of Sciences of the United States of America,* 107(28), pp. 12687-12691.

Yousefzadeh, M. J., Schafer, M. J., Noren Hooten, N., Atkinson, E. J., Evans, M. K., Baker, D. J., Quarles, E. K., Robbins, P. D., Ladiges, W. C., LeBrasseur, N. K. and Niedernhofer, L. J. (2018) 'Circulating levels of monocyte chemoattractant protein-1 as a potential measure of biological age in mice and frailty in humans', *Aging Cell,* 17(2), pp. 7.

Zarbock, A., Bishop, J., Muller, H., Schmolke, M., Buschmann, K., Van Aken, H. and Singbartl, K. (2010) 'Chemokine homeostasis vs. chemokine presentation during severe acute lung injury: the other side of the Duffy antigen receptor for chemokines', *American Journal of Physiology-Lung Cellular and Molecular Physiology,* 298(3), pp. L462-L471.

Zhang, C., Li, H. W. and Han, R. Z. (2020) 'An open-source video tracking system for mouse locomotor activity analysis', *Bmc Research Notes,* 13(1), pp. 6.

Zhang, J. and De Koninck, Y. (2006) 'Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury', *Journal of Neurochemistry,* 97(3), pp. 772-783.

Zhang, J., Patel, L. and Pienta, K. J. (2010a) 'Targeting chemokine (C-C motif) ligand 2 (CCL2) as an example of translation of cancer molecular biology to the clinic', *Prog Mol Biol Transl Sci,* 95, pp. 31-53.

Zhang, J., Shi, X. Q., Echeverry, S., Mogil, J. S., De Koninck, Y. and Rivest, S. (2007) 'Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain', *Journal of Neuroscience,* 27(45), pp. 12396-12406.

Zhang, J. M. and An, J. (2007) 'Cytokines, inflammation, and pain', *Int Anesthesiol Clin,* 45(2), pp. 27-37.

Zhang, L., Tan, J. Y., Jiang, X. P., Qian, W. W., Yang, T., Sun, X. J., Chen, Z. H. and Zhu, Q. W. (2017) 'Neuron-derived CCL2 contributes to microglia activation and neurological decline in hepatic encephalopathy', *Biological Research,* 50.

Zhang, X., Zhong, W., Brankačk, J., Weyer, S. W., Müller, U. C., Tort, A. B. and Draguhn, A. (2016) 'Impaired theta-gamma coupling in APP-deficient mice', *Sci Rep,* 6, pp. 21948.

Zheng, C., Bieri, K. W., Hsiao, Y. T. and Colgin, L. L. (2016) 'Spatial Sequence Coding Differs during Slow and Fast Gamma Rhythms in the Hippocampus', *Neuron,* 89(2), pp. 398-408.

Zhou, Y., Tang, H. M., Liu, J. U., Dong, J. and Xiong, H. G. (2011) 'Chemokine CCL2 modulation of neuronal excitability and synaptic transmission in rat hippocampal slices', *Journal of Neurochemistry,* 116(3), pp. 406-414.

Zink, M. C., Coleman, G. D., Mankowski, J. L., Adams, R. J., Tarwater, P. M., Fox, K. and Clements, J. E. (2001) 'Increased macrophage chemoattractant protein-1 in cerebrospinal fluid precedes and predicts simian immunodeficiency virus encephalitis', *Journal of Infectious Diseases,* 184(8), pp. 1015-1021.