An *in vivo* electrophysiological and computational analysis of hippocampal synaptic changes in the Alzheimer’s disease mouse

A thesis submitted to
The University of Manchester
for the degree of
Doctor of Philosophy
in the Faculty of Life Sciences.

2015

*Author:*
Daniel Squirrell

*Supervisors:*
Dr. John Gigg
Dr. Marcelo Montemurro
# Contents

<table>
<thead>
<tr>
<th>List of Figures</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>8</td>
</tr>
<tr>
<td>Declaration &amp; Copyright statement</td>
<td>9</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>10</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>11</td>
</tr>
<tr>
<td><strong>1 Introduction</strong></td>
<td>13</td>
</tr>
<tr>
<td>1.1 Overview</td>
<td>13</td>
</tr>
<tr>
<td>1.2 Alzheimer’s disease</td>
<td>14</td>
</tr>
<tr>
<td>1.2.1 Alzheimer’s disease pathology</td>
<td>15</td>
</tr>
<tr>
<td>1.2.2 Production and distribution of Alzheimer’s disease related pathology</td>
<td>16</td>
</tr>
<tr>
<td>1.2.3 Models of Alzheimer’s disease</td>
<td>19</td>
</tr>
<tr>
<td>1.2.4 3xTgAD mouse model of Alzheimer’s disease</td>
<td>21</td>
</tr>
<tr>
<td>1.3 The Hippocampus</td>
<td>24</td>
</tr>
<tr>
<td>1.3.1 Field CA1 and subiculum</td>
<td>28</td>
</tr>
<tr>
<td>1.3.2 Synaptic plasticity</td>
<td>30</td>
</tr>
<tr>
<td>1.3.3 Assessment of neuronal function of Alzheimer’s disease mice</td>
<td>32</td>
</tr>
<tr>
<td>1.4 Neuronal oscillations and their role in memory</td>
<td>36</td>
</tr>
<tr>
<td>1.4.1 Types of oscillations</td>
<td>37</td>
</tr>
<tr>
<td>1.4.2 What is sleep and what function does it serve?</td>
<td>39</td>
</tr>
<tr>
<td>1.4.3 The physiological relevance of network oscillations</td>
<td>40</td>
</tr>
<tr>
<td>1.5 Alzheimer’s disease associated changes in network oscillations</td>
<td>46</td>
</tr>
<tr>
<td>1.5.1 The use of EEG in Alzheimer’s disease</td>
<td>46</td>
</tr>
<tr>
<td>1.5.2 Coherence and information analysis</td>
<td>48</td>
</tr>
<tr>
<td>1.6 Urethane as the anaesthetic of choice for acute recordings in rodents</td>
<td>49</td>
</tr>
<tr>
<td>1.7 Specific research aims</td>
<td>52</td>
</tr>
<tr>
<td>1.8 Alternative format structure</td>
<td>53</td>
</tr>
<tr>
<td>1.8.1 Paper 1</td>
<td>53</td>
</tr>
<tr>
<td>1.8.2 Paper 2</td>
<td>53</td>
</tr>
</tbody>
</table>
## 2 Methods

2.1 Animals ............................................ 56
2.2 Anaesthesia and surgery ............................... 57
2.3 Electrode placement .................................... 58
2.4 Spontaneous recordings and data acquisition ...... 58
2.5 Stimulation protocol .................................. 59
  2.5.1 Insertion of stimulating electrode ................. 59
  2.5.2 Assessment of functional neuronal connectivity 61
  2.5.3 Assessing short-term plasticity using paired-pulse stimulation .... 61
  2.5.4 Low-frequency train stimulation .................... 61
  2.5.5 Long-term potentiation ............................ 61
2.6 Perfusion and storage of brain tissue ............... 62
2.7 Overview of experimental protocol .................... 62
2.8 Histology .............................................. 62
2.9 Data analysis ........................................ 63
  2.9.1 Electrode localisation ............................... 63
  2.9.2 Channel selection for further analysis ............. 65
  2.9.3 Analysis of the stimulation data .................. 66
  2.9.4 Current Source Density (CSD) Analysis ............. 68
  2.9.5 State separation ................................... 71
  2.9.6 Power analysis .................................... 75
  2.9.7 Transfer entropy analysis .......................... 75
2.10 Image processing and cell counting ................... 79

## 3 Paper 1

3.1 Abstract ........................................... 81
3.2 Introduction ......................................... 82
3.3 Materials and Methods ................................ 84
  3.3.1 Animals .......................................... 84
  3.3.2 Anaesthesia and surgery .......................... 84
  3.3.3 Stimulation ........................................ 86
  3.3.4 Data acquisition and analysis ...................... 87
  3.3.5 Current source density analysis .................... 88
  3.3.6 Image processing and cell counting ................. 89
3.4 Results ............................................... 89
  3.4.1 Similar response profiles between all control and 3xTgAD mice .... 89
  3.4.2 Changes in basal synaptic connectivity .............. 90
  3.4.3 Paired-pulse facilitation deficits are apparent from 3 months in the 3xTgAD mouse .................. 91
  3.4.4 Differences in facilitation are not due to a change in the rate of ascending phase of the evoked EPSP .......... 93
3.4.5 Responses to stimulus trains ............................................... 93
3.4.6 Current source density to 5Hz low-frequency stimulation reveals intact CA1→subiculum connectivity .............................................. 97
3.4.7 Current source density to 10Hz low-frequency stimulation reveals intact CA1→subiculum connectivity .............................................. 100
3.4.8 3xTgAD mice are unable to express long-term potentiation ........ 100
3.4.9 No neuronal cell loss with age or AD-like pathology progression ... 102
3.5 Discussion ........................................................................... 103

4 Paper 2 ................................................................................. 111
4.1 Abstract .............................................................................. 111
4.2 Introduction .......................................................................... 112
4.3 Materials and Methods ............................................................. 114
  4.3.1 Animals ........................................................................... 114
  4.3.2 Anaesthesia and surgery ....................................................... 114
  4.3.3 Spontaneous recordings and data acquisition ......................... 115
  4.3.4 Perfusion and storage of brain tissue ..................................... 116
  4.3.5 Electrode localisation .......................................................... 116
  4.3.6 Electrophysiological state filtering ........................................ 117
  4.3.7 Band-pass filtering for delta, theta and slow-wave ripple oscillations 117
  4.3.8 Power analysis ................................................................... 118
  4.3.9 Cross-covariance analysis ..................................................... 118
  4.3.10 Transfer entropy analysis .................................................... 118
4.4 Results .................................................................................. 120
  4.4.1 Urethane anaesthesia induces reliable and stable sleep-like network oscillations ............................................................. 121
  4.4.2 Changes in the power of subcortical rhythms with age and AD-like pathology ................................................................. 121
  4.4.3 Differences in the cross-covariance of theta rhythms ................ 124
  4.4.4 Deficits in the information carrying capacity of delta state dominant signals in 3-month 3xTgAD mice ........................................... 124
  4.4.5 Deficits in 3-month 3xTgAD mice during delta dominant periods are predominately due to changes in the information carrying capacity of SWRs ................................................................. 129
  4.4.6 Deficits in the information capacity of SWR oscillations are not due to a lack of SWR oscillation in 3xTgAD mice .............................. 130
  4.4.7 Deficits become apparent in the filtered theta signals in the 3xTgAD mouse by 6 months ........................................................... 130
  4.4.8 3xTgAD mice show deficits in the slow-wave component of delta rhythms at 9 months ............................................................ 135
4.5 Discussion ............................................................................. 135
5 Discussion

5.1 Overview

5.2 Changes in synaptic connectivity in the CA1-subicular network

5.3 Age and disease related changes in the spectral power of spontaneous network oscillations

5.4 Deficits in the information carrying capacity during spontaneous network oscillations

5.5 Limitations of the study

5.6 Conclusions and future work

References
List of Figures

1.1 Alzheimer’s disease pathology ............................................. 17
1.2 Amyloid Cascade Hypothesis .............................................. 18
1.3 Hippocampal connectivity ................................................. 27
1.4 Electrophysiological recordings in the 3xTgAD mouse ................. 34
1.5 Hasselmo model of hippocampal encoding and retrieval of memories during theta rhythms ........................................... 42
1.6 Theta phase precession ..................................................... 44
2.1 Placement of recording electrodes ........................................ 59
2.2 Insertion of stimulating electrode and typical subicular LFP response profile ......................................................... 60
2.3 Confirmation of electrode localisation .................................... 64
2.4 Example of subicular evoked EPSP and PPF ............................ 65
2.5 Obtaining measurements from subicular EPSPs ...................... 67
2.6 Illustration of CSD profiles through the stimulation train ............. 70
2.7 Common spatial components present in CSD profiles ............... 70
2.8 Illustration of LFP and FFT pre- and post state separation ........... 72
2.9 Determination of state-dominant periods in LFP signal ............ 73
2.10 State separation based on the relative dominance versus other rhythms ................................................................. 74
2.11 Example of individual and region-specific average FFTs ........... 76
3.1 Subicular evoked response profiles are similar between control and 3xTgAD mice ................................................................. 90
3.2 Current input-output response relationship ............................ 92
3.3 Early changes in short-term plasticity in the 3xTgAD mouse ....... 94
3.4 No differences are apparent in the relative speed of the ascending phase of the evoked EPSP during 50ms PPS ......................... 95
3.5 Low-frequency repetitive stimulation reveals synaptic deficits in 3xTgAD mice during the early stages of AD-like pathology ............... 98
3.6 Current source density analysis of responses during 5Hz train ...... 99
3.7 Current source density analysis of subicular responses to 10Hz stimulation of CA1 ................................................................. 101
3.8 Early deficits in LTP induction in the 3xTgAD mouse .................. 102
3.9 Control and 3xTgAD mice do not suffer from overall cell loss as a function
of age or disease-like pathology, respectively. .............................................. 103

4.1 Illustration of experimental preparation ....................................................... 122
4.2 Age and genotype related changes in network oscillations. ........................... 125
4.3 Cross-covariance between CA1 and subiculum for delta and theta rhythms. .... 126
4.4 Deficits in the flow of information during delta-dominant, non-filtered periods
in the 3-month 3xTgAD mouse ................................................................. 128
4.5 SWRs are a key component of the delta-dominant, non-filtered signal involved
in the transfer of information in the CA1-subicular pathway in 3
month mice .................................................................................................. 131
4.6 Autocorrelation analysis of SWRs in 3 month mice ....................................... 132
4.7 Regional power of SWR oscillations in 3 month mice ..................................... 132
4.8 Increased flow of information during theta filtered periods in the 6-month
3xTgAD mouse .............................................................................................. 134
4.9 Deficits in the flow of information during delta filtered periods in the 9-month
3xTgAD mouse .............................................................................................. 136
4.10 Transfer entropy is similar between genotypes during periods of theta in 3
and 9 month mice .......................................................................................... 137
Alzheimer’s disease (AD) is a neurodegenerative disorder resulting in the decline of cognitive function, memory formation and retrieval, and abrupt changes in personality. Damage to brain networks occur during prodromal stages of AD, prior to the development of clinical symptoms of dementia. Further characterising this state and identifying reliable biomarkers for early detection are priorities in AD research.

I characterised neuronal changes within the dorsal CA1 and subiculum regions of the hippocampal formation (HF) in the well-characterised 3xTgAD mouse model of AD. These regions are well-established sites for early neurodegeneration in both AD patients and AD animal models. We inserted multi-electrode recording arrays into CA1 and subiculum of urethane anaesthetised 3xTgAD mice and recorded spontaneous local field potential activity. Using traditional and novel information theoretic approaches, I determined the information carrying capacity of the CA1-subiculum network during different network rhythms, and how this altered with age and AD-like pathology. A bipolar stimulating electrode was inserted into CA1, allowing the assessment of synaptic integrity between CA1 and subiculum.

Results showed that synaptic and network changes occur in CA1 and subiculum during the early stages of AD-like pathology and correlates with the development of intracellular beta-amyloid. There is a progressive breakdown in synaptic facilitation as early as 3 months in the 3xTgAD mouse. These data support an advanced ageing-like phenotype in AD model mice, with an enhanced age/pathology-dependent breakdown in neuronal communication compared to age-matched controls. In agreement with other studies, 3xTgAD mice demonstrate evidence of pathology-related changes in the network rhythms of the HF. 3xTgAD mice show an increase in the power of alpha and beta rhythms, and a concurrent reduction in the power of delta oscillations. Application of novel information theoretic techniques results in a breakdown in the information carrying capacity of the hippocampal system. This deficit manifests as a reduction in information flow during delta-dominant periods of EEG rhythms, with a specific reduction during slow-wave ripple activity. This change in neuronal communication correlates with the onset of memory-retention/consolidation deficits. These network changes are complex, with alterations in the information carrying capacity of the system during theta rhythms at 6 months, and during slow-wave components by 9 months in the 3xTgAD mouse.

This study provides the first evidence of an early and progressive decline in neuronal connectivity and communication that correlates with changes in cognition in the 3xTgAD mouse. Application of novel analytical techniques to multi-site EEG recording revealed early and measureable changes in information processing during the onset of AD-like pathology. These are important new biomarkers for early AD characterisation.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright statement

i The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the “Copyright”) and s/he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii The ownership of certain Copyright, patents, designs, trade marks and other intellectual property (the “Intellectual Property”) and any reproductions of copyright works in the thesis, for example graphs and tables (“Reproductions”), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=487), in any relevant Thesis restriction declarations deposited in the University Library, The University Library’s regulations (http://www.manchester.ac.uk/library/aboutus/regulations) and in The University’s Policy on Presentation of Theses.
Acknowledgements

I would like to take this opportunity to thank my supervisors Dr. John Gigg and Dr. Marcelo Montemurro for their continued support throughout my PhD. Your continued help, encouragement and friendship has made completing my PhD a surprisingly enjoyable experience.

I would also like to thank Dr. Sarah Fox for her continued support and advice during my many Matlab nightmares, Dr. Fiona Burrows for her patience whilst passing on her invaluable tracheotomy skills, and Maria Constantinou for her programming wizardry. In addition to your continuous academic help, I would like to thank you for all of the conversations and numerous coffee breaks that kept me going over the last few years. I would also like to thank the Lucas and Brown lab for their support and company during those long days in the lab. I would like to pay particular thanks to my Nan for her support, generosity, kindness, and the never-ending supply of cake. I would also like to express my thanks to Linda and Nigel for their kindness and generosity, and for providing a home in the sunny south during those much needed breaks. Finally, I thank my partner Samantha for also being there for me and making sure that I get out of bed in time every morning. Without your patience, care, kindness and support I can guarantee that this would not have been possible.

I would like to thank both Costa and Starbucks coffee for providing me with fuel throughout my PhD. I still remember that moment when I collected my 50th star and became a ‘Starbucks King’; making that supply of coffee that little bit easier on my wallet.

I also acknowledge the Alzheimer’s Society for funding this project and for inviting me to their annual events and conferences. It’s been a pleasure to be part of their research team; helping to lead the fight against dementia.
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>Aβ</td>
<td>Beta-amyloid</td>
</tr>
<tr>
<td>CA1</td>
<td>Cornu ammonis field 1</td>
</tr>
<tr>
<td>CA3</td>
<td>Cornu ammonis field 3</td>
</tr>
<tr>
<td>CSD</td>
<td>Current source density</td>
</tr>
<tr>
<td>DG</td>
<td>Dentate gyrus</td>
</tr>
<tr>
<td>EC</td>
<td>Entorhinal cortex</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>EPSP</td>
<td>Excitatory post-synaptic potential</td>
</tr>
<tr>
<td>fEPSP</td>
<td>Field excitatory post-synaptic potential</td>
</tr>
<tr>
<td>FFT</td>
<td>fast Fourier transform</td>
</tr>
<tr>
<td>FTD</td>
<td>Frontotemporal dementia</td>
</tr>
<tr>
<td>HC</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>HF</td>
<td>Hippocampal formation</td>
</tr>
<tr>
<td>HFS</td>
<td>High frequency stimulation</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>I/O</td>
<td>Input/Output</td>
</tr>
<tr>
<td>IPSP</td>
<td>Inhibitory post-synaptic potential</td>
</tr>
<tr>
<td>LFP</td>
<td>Local field potential</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>MEA</td>
<td>Multi electrode array</td>
</tr>
<tr>
<td>MI</td>
<td>Mutual information</td>
</tr>
<tr>
<td>NFT</td>
<td>Neurofibrillary tangle</td>
</tr>
<tr>
<td>PC</td>
<td>Place cell</td>
</tr>
<tr>
<td>PP</td>
<td>Perforant pathway</td>
</tr>
<tr>
<td>PPF</td>
<td>Paired-pulse facilitation</td>
</tr>
<tr>
<td>PPS</td>
<td>Paired-pulse stimulation</td>
</tr>
<tr>
<td>PS1/PS2</td>
<td>Presenilin 1/2</td>
</tr>
<tr>
<td>PV</td>
<td>Parvalbumin</td>
</tr>
<tr>
<td>SWS</td>
<td>Slow-wave sleep</td>
</tr>
<tr>
<td>SWR</td>
<td>Slow-wave ripple</td>
</tr>
<tr>
<td>TA</td>
<td>Temporoammonic pathway</td>
</tr>
<tr>
<td>TE</td>
<td>Transfer entropy</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

1.1 Overview

Alzheimer’s disease (AD) is a neuronal degenerative condition resulting in the gradual deterioration of cognitive function. With no current therapeutic for AD, there is a critical need to develop our understanding of the disease processes through scientific research. Degeneration is commonly found in various regions throughout the brain including, memory and emotional related regions within the limbic system and association areas. This neuronal degeneration is commonly accompanied with aggregated beta-amyloid (Aβ) and neurofibrillary tangle pathology (NFT) (Selkoe, 2011).

Currently, diagnosis of AD focuses on the clinical presentation of symptoms, and is typically based on the cognitive capacity of the patient. However, clinical diagnosis typically requires a combination of features, including cognitive impairment that interferes with day-to-day activities, evidence of gradual and progressive decline in memory performance, patient history assessment, assessment of impairments through the use of a mini-mental state examination, and at least two separate cognitive impairments (McKhann et al., 1984, 2011). Therefore there are limitations with the current clinical requirements for diagnosis, as patients commonly experience mild alterations in cognitive performance prior to receiving an AD diagnosis. Therefore, it is evident that we need to further investigate and develop our understanding of the neuronal changes that take place during the early stages of the disease, prior to overt clinical symptoms (McKhann et al., 1984; Pugh et al., 2007; Simic et al., 2009; Budson and Solomon, 2012).

Recent research shows that AD and its associated pathology affects many aspects of neuronal function and that AD pathology is commonly found in regions involved
in the generation of network oscillations, including the brainstem and the entorhinal cortex (EC) (Simic et al., 2009; Stranahan and Mattson, 2010; Brown et al., 2012). Since neuronal oscillations result in inter-regional synchrony, it is thought that this provides a ‘window of opportunity’ for regions to communicate and transfer information. It would therefore be expected that any alterations in the entrainment of neuronal populations through inter-regional coherence could result in global changes in the gating of information flow through the brain (Fries, 2005).

It is clear that there is a need to conduct further research into understanding the mechanisms and network changes induced by AD. To help tackle this issue, dementia research can utilise a variety of mouse models of AD, many of which carry genes known to be associated with human AD. These models aim to reproduce disease mechanisms and AD-related phenotypes, such as altered cognitive capacity, plaque and tangle pathology, synaptic changes and alterations in electroencephalogram (EEG) signals (Brenner et al., 1988; Thal et al., 2002; Bilkei-Gorzo, 2014). Therefore, there is a clear benefit in making use of these murine models in dementia research in order to further develop our understanding of the disease.

1.2 Alzheimer’s disease

Alzheimer’s disease is the most common of the four types of dementia and is currently diagnosed solely based upon clinical presentation of symptoms and later confirmed post-mortem by the presence of Aβ plaques and hyperphosphorylated tau in brain tissue (Grossman et al., 2006; Möller and Graeber, 1998; Harrison, 2013). However, the presence of plaques is not a reliable marker of AD in all cases (Braak and Braak, 1991). Indeed, the widely used Braak and Braak stages for AD are characterised by the distribution of tau tangles rather than plaques (Braak and Braak, 1991).

There is growing evidence to suggest that AD-related pathological changes occur prior to overt cognitive abnormalities. As a result, it is likely that the current method of diagnosis (see earlier) is a) unreliable, and b) typically results in a diagnosis after the development of impairments, which may be too late to help alleviate symptoms. This could help to explain the poor performance of drugs when they are advanced to clinical trials as they are typically administered to patients who have advanced to full AD and therefore are likely to have global changes in the neuronal network (Budson and Solomon, 2012). Thus, there is a good case for these drugs to be re-validated against markers of the disease in patients at the ‘prodromal’ stages of the disease, prior to clinical symptoms of any cognitive impairment. However, these prodromal markers still need to be identified and validated (Karran et al., 2011).
1.2. Alzheimer’s disease

Whilst we know of various risk factors for developing the disease, age is by far the biggest, with the majority of AD patients diagnosed over the age of 65 years. Whilst this is true for sporadic forms of AD, representing the majority of AD cases, in the small minority of cases (3-5%) there are direct genetic links, typically resulting in the early-onset (familial) AD. Due to a combination of a lack of treatment/cure for AD, coupled with advances in medical research leading to an ever-ageing population, the prevalence of the disease is forecast to double every 20 years (Ferri et al., 2005). Clearly, this will represent a huge financial and emotional burden to families, carers and governmental resources, and so it is vital that we lead pioneering research in this field in search of preventative and palliative strategies.

It is possible that there could be substantial benefit to conducting research on the early ‘prodromal’ or AD-related mild cognitive impairment (MCI) stages of the disease in order to develop a diagnostic tool for early intervention and assessment of candidate therapeutics. We are currently able to address this through use of animal models of disease. Genetically modified mouse models typically express human transgenes, known to be associated with AD, and usually affect the Aβ processing pathway. Whilst we can appreciate that these mice have not ‘developed’ AD, they do mimic the human AD phenotype and allow us to assess how neuronal changes occur as a function of age and disease progression (Spires-Jones and Knafo, 2012; Bilkei-Gorzo, 2014).

1.2.1 Alzheimer’s disease pathology

Alzheimer’s disease is classified post-mortem by the presence of two hallmark pathological lesions; Aβ plaques and NFTs (Selkoe, 2011). Both tau (a protein involved in the production of NFTs) and Aβ, when at physiological levels, are normal constituents in neuronal cells and play an important role in the neuronal structure and function. However, when their production and/or clearance become disrupted it typically leads to misprocessing and a toxic build up of pathology which is often found in AD post mortem brain tissue (see Figure 1.1) (Duyckaerts et al., 2009).

Beta-amyloid accumulation usually results due to mis cleavage and processing of the amyloid precursor protein (APP). Genetic variations in the APP and presenilin (PS) proteins have been shown to result in genetic predispositions to developing AD. APP is a type I membrane-associated protein which undergoes proteolytic cleavage by the α or β-secretase pathway (LaFerla, 2002). The processing of APP ultimately determines the length of the resulting Aβ fragments, where processing by the α-secretase enzyme results in non-amyloidogenic fragments, whilst processing through the β-secretase pathway typically results in the production of amyloidogenic
fragments. This miscleavage leads to misfolding of the fragments of the APP protein, resulting in the production of Aβ_{1-42}. Isoforms of Aβ at this length have a greater propensity to aggregate and form Aβ oligomers which develop into extracellular plaques/fibrils (LaFerla, 2002).

NFTs are formed due to a different process. In this instance, the microtubule associated protein tau becomes hyperphosphorylated, resulting in the loss of its dynamic structure. Under normal physiological conditions tau plays an important role in the assembly and stabilisation of microtubules, and as such is vital for the maintenance of the structural integrity of neurons (Crowther and Goedert, 2000). In addition, under normal conditions the tau protein is only found to be located within the axons of neurons, however in AD it is typically found to migrate to other regions, such as dendrites and cell bodies (Crowther and Goedert, 2000). The loss of its stability in AD results in the structure of the neuron becoming compromised, resulting in shrinkage and cell death.

As well as the two key hallmark lesions found in AD brain tissue, there are other important pathological processes that take place during the disease, such as up-regulation of inflammation, oxidative stress, mitochondrial dysfunction and changes in protein sorting.

### 1.2.2 Production and distribution of Alzheimer’s disease related pathology

Accumulation of AD pathology occurs in the CNS of patients of both sporadic and familial forms of AD and typically accumulates in a very well defined temporal and spatial pattern (Nelson et al., 2012). This temporal-spatial relationship differs between Aβ and tau pathology, with Aβ developing firstly in the cortex and later in the hippocampus (HC) following a centripetal pattern, whilst NFTs first develop in the HC and later in the cortex following a centrifugal pattern (Thal et al., 2000, 2002). Therefore, Aβ accumulates first in neocortex and progresses over time to the trans-entorhinal region, entorhinal cortex (EC), CA1, subiculum, amygdala and brainstem nuclei. Whilst tau pathology is initially found within the trans-entorhinal region, followed by EC, HC, and the temporal, parietal and occipital cortex (Braak and Braak, 1991; Mastrangelo and Bowers, 2008).

The correlation between the spatio-temporal accumulation of pathology with changes in cognitive function has been debated for many years. The amyloid cascade hypothesis (see Figure 1.2) has recently been questioned as new theories suggest that the development of tau pathology more strongly correlates with cognitive decline (Arriagada et al., 1992). Recent work shows that the distribution of NFTs and
Figure 1.1. Alzheimer’s disease pathology. Immunohistochemical detection of neurofibrillary tangles in the occipital lobe (A and B). Scale bars for A and B are 100µm and 150µm, respectively. Immunohistochemical detection of Aβ plaques in the neocortex (C), neostriatum (D), and molecular layer of the cerebellum (E). Scale bars represent 500µm. Structural MRI scans relative to a scan from a cognitively normal patient (F) show progressive neuronal atrophy in a patient with MCI (G) and AD (H). (Images adapted from Montine et al. (2012) and Vemuri and Jack (2010).)
synaptic loss correlates well with cognitive decline in AD and develops prior to overt Aβ plaque pathology.

Figure 1.2. Amyloid Cascade Hypothesis Cleavage of the amyloid precursor protein can occur via the α- (left) or β- (right) secretase pathways. Cleavage via α-secretase (left of APP in figure) results in the production of a soluble APP fragment. However, cleavage via β-secretase and subsequently cleavage γ-secretase results in the release of the amyloidogenic forms of Aβ. The formation of the amyloidogenic Aβ1−42 fragments are found to correlate with the onset of cognitive and neuronal deficits. The Aβ monomers are more likely to aggregate and subsequently form oligomers and during later stages develop into insoluble Aβ fibrils/plaques (Hardy and Selkoe, 2002).

However, although there is a strong link between tau pathology and cognitive decline, the amyloid cascade hypothesis still remains the most favourable theory for disease development and its progression (Hardy and Higgins, 1992). The amyloid cascade hypothesis suggests that declining cognitive performance and neuronal degeneration is due to a change in the processing of APP, resulting in a neurotoxic build up of Aβ protein. It also proposes that this process initiates all other associated pathology accumulation and, therefore, that Aβ plays a causative role in AD progression. In support of this theory, all known genetic links and predispositions to AD are found to affect APP processing, resulting in the build-up of aberrant Aβ protein. All variations in the mutations of the APP and PS1/2 are found to result in the system favouring the development of amyloidogenic Aβ1−42 (Hardy and Higgins, 1992; Hardy and Selkoe, 2002). Furthermore, studies in mice that solely contain mutations resulting in the development of NFTs are found not to develop Aβ pathology, which provides further evidence that tau pathology is generated independently of the APP pathway (Crowther and Goedert, 2000; Winton et al., 2011; Wolfe, 2012; Spillantini and Goedert, 2013).

Whilst the amyloid cascade hypothesis still remains the most common theory
for AD pathology, arguments against this include; a) Aβ plaques do not correlate absolutely with cognitive decline and b) extracellular Aβ plaques are commonly found in the brain of healthy individuals with a distribution and structure similar to those found in AD tissue (Skaper, 2012; Davis et al., 1999; Haroutumian et al., 1998).

Importantly, more recent work provides support for a causative effect of beta-amyloid, rather than tau pathology and therefore provides weight to the amyloid-cascade hypothesis. These studies propose that it is in fact the early intracellular soluble oligomer forms of Aβ that correlate with cognitive decline rather than the insoluble extracellular Aβ plaques. This intracellular form of aberrant Aβ not only correlates with the onset of cognitive decline but also results in altered synaptic communication (Klyubin et al., 2012; Oddo et al., 2003; Billings et al., 2005). Studies also suggest that NFT accumulation, whilst correlated with cognitive decline, is in fact a function of the normal ageing process, which becomes exacerbated by intracellular accumulation of aberrant Aβ (Götz, 2001; Lewis et al., 2001; Swerdlow, 2012). However, whilst studies have indicated a potential causative effect of Aβ in AD, to date all Aβ immunotherapy clinical trials have failed to progress past phase III due to unacceptable adverse events and/or poor clinical efficacy (Lannfelt et al., 2014).

In addition, these conclusions must be viewed with caution due to the mechanisms by which genetic expression is controlled. It is important to consider the limitations associated with the direct comparison with mouse models and the issues associated with transgenesis/promoters. For example, pathology expression is under tight control of the Thy1.2 promoter in the 3xTgAD mouse and therefore they are designed in a way that will result in similar pathological expression to that found in humans. As such, whilst murine models represent a valuable method to help to facilitate the mechanisms/pathology-related neuronal changes, we must be cautious when directly comparing the spatial/temporal progression of pathology with the Braak and Braak staging in AD (Oddo et al., 2003; Braak and Braak, 1991).

### 1.2.3 Models of Alzheimer’s disease

Clearly there is a lack of understanding of the mechanisms of the disease, and therefore there is a requirement of dementia research to invest more time and resources into animal models of AD in order to further our understanding of the disease mechanisms and progression. The characterisation of prodromal markers for the disease is of paramount importance for early clinical intervention. Thus, it is vital for us to change the focus of research to understand changes in neuronal networks during
the early stages of AD in order to address the issue relating to the historically poor performance of drugs that have been advanced to clinical trials. Although there are obvious limitations associated with the use of animal models, in that they all model the familial forms of AD, and that, apart from the recent rat model, fail to develop tau pathology, it is to date the best and most appropriate method to develop our understanding (Cohen et al., 2013). The main aim of transgenic AD models is to produce an animal that mimics the mechanisms and closely matches the disease phenotypes that are known to be associated with the human form of the disease.

Whilst most AD mouse models rely on genetic manipulation, it is important to note that genetically linked (familial) AD only accounts for \( \sim 2-5\% \) of cases. Therefore these models represent those AD patients who have absolute genetic vulnerability, whereas sporadic forms of AD develop due to a combinatorial effect of a large number of risk factors (Tanzi, 2012; Götz et al., 2004). Genetically linked, familial forms of AD, typically result in early onset of the disease, with the majority of patients <65 years old, whilst sporadic forms with few known genetic links typically results in late onset of the disease (Roses, 1994; Tanzi, 2012). Although there are two distinct subsets of patients, all cases are found to present the same cognitive and pathological phenotypes and, therefore, validate the use of genetically modified animals in the study of AD. In addition, 2-5% of AD cases still accounts for a large number of patients and will remain constant in absolute terms, and decrease in relative terms as a result of an ageing population. Therefore, understanding the mechanisms of, at least, familial AD is a big advance in developing our understanding of the disease.

One of the most prevalent genetic mutations is found in the gene encoding APP, with around 20 known genetic variations. The most common of these are the London and Swedish mutations, with all variations known to result in the over-production of \( \text{A}^\beta_{1-42} \). However, the most common genetic predisposition lies in the genes encoding PS1/PS2. Mutations in these result in a change in the APP processing pathway, with an increase in gamma-secretase activity. Again, this results in the over-production of the amyloidogenic form of \( \text{A}^\beta \). Due to the relative ease of genetic manipulation of the mouse genome (and their true breeding across generations) we have been able to exploit these transgenes by inserting them singly or in combination to replicate the AD phenotype in murine models. Whilst the majority of AD models have made use of the relatively easy genetic manipulation of mice, researchers have recently developed a rat model of AD, which unlike mouse models, develops the full \( \text{A}^\beta \) and tau pathological phenotype without the addition of a tau transgene (Cohen et al., 2013).
Due to the combinations of transgenes, AD mouse models typically fall into four categories; 1) APP, 2) PS1 or PS2, 3) Tau or 4) any combination of these transgenes, such as in the TASTPM and 3xTgAD mice (for review see Hall and Roberson, 2012). Whilst all of these mice (excluding Tau mutations) result in an AD phenotype, different models show differences in the temporal-spatial development of pathology and differing rates of cognitive decline due to the combinatorial effect of introducing multiple variations.

The first transgenic model was created in 1995 and was introduced in response to the development of the amyloid-cascade hypothesis, exploiting mutations in the APP gene and therefore affect Aβ production (Hardy and Allsop, 1991; Games et al., 1995). Following the introduction of genetic variations involved in the processing of APP, various other models soon followed, some introducing multiple variations of the same gene, such as in the 5xFAD mouse (Oakley et al., 2006), and other introducing combinations of genes e.g. APP and PS1 in the TASTPM mouse (Howlett et al., 2008).

The introduction of both the APP and PS1/PS2 genes results in misprocessing of the APP, resulting in an overproduction of Aβ1-42. Whilst mutations in the APP gene are found to solely produce increased levels of Aβ, variations in the PS gene are also known to affect calcium regulation in neurons and, therefore, have the potential to alter synaptic function (Duff et al., 1996; Citron et al., 1997; Chui et al., 1999; Sabbagh et al., 2013).

Whilst there is no known genetic link to tau pathology in AD, one limitation to the majority of AD mouse models is the lack of spontaneous tau pathology in mice carrying mutations in the APP and/or PS transgenes, with changes only found in Aβ pathology (Holcomb et al., 1998; Oakley et al., 2006). However, there are known genetic links to tau production in other dementias, such as in frontotemporal dementia (FTD). Therefore, the ideal candidate mouse model of AD would express the full behavioural and pathological phenotype. In response to this requirement, Oddo and colleagues developed and introduced the 3xTgAD mouse by combining mutations involved in APP processing (APP<sub>SWE</sub> and PS1<sub>M146V</sub>) and a tau transgene from FTD (Tau<sub>P301L</sub>) (Oddo et al., 2003).

### 3xTgAD mouse model of Alzheimer’s disease

The 3xTgAD mouse is one of the only murine models to combine three transgenes in order to produce a model that displays the full pathological and behavioural phenotype present in human cases of AD (Oddo et al., 2003). Whilst there has been little research assessing the electrophysiological changes associated with the
disease and its progression *in vivo* in the 3xTgAD and all other models of AD there has been limited research conducted *in vitro* (Oddo et al., 2003). To date, studies have found evidence of electrophysiological changes in these mice in mono- and multisynaptic pathways, including in the hippocampal re-entrant pathway at 4-6 months (Oddo et al., 2003; Davis et al., 2014). Interestingly, these synaptic changes correlate with the onset of spatial and episodic-like memory deficits, which is one of the first detectable cognitive impairments in human AD (Billings et al., 2005; Oddo et al., 2003; Davis et al., 2013b,a). Importantly, studies have confirmed that 3xTgAD mice are functionally, behaviourally and pathologically ‘normal’ at birth in comparison to their age-matched control counterparts. Therefore, we can assume that any functional and behavioural changes are associated with the onset and development of AD-like disease pathology (Billings et al., 2005; Clinton et al., 2007; Oddo et al., 2003).

### Pathological phenotype

To date, the most comprehensive pathological study conducted on the 3xTgAD mouse has been performed by Mastrangelo and Bowers (2008). In their study they characterised the spatial and temporal distribution of several pathological markers, including, APP, A$_\beta_{1-42}$, hyperphosphorylated tau, paired-helical filaments and microglial activation in mice aged 2-26 months (Mastrangelo and Bowers, 2008). In support of others studies, they also provided supporting evidence that this model is pathologically ‘normal’ at birth and shows a lack of any AD-related pathology. Pathology first becomes apparent at 2 months, revealed by diffuse staining of 6e10 antibody (a marker for both APP and A$_\beta$) in the motor cortex, amygdala and CA1/CA3 region of the HC. Interestingly, they found evidence for intracellular forms of A$_\beta$ in the CA1-Subiculum border, which is similar to pathology reported in human forms of MCI, a precursor of AD-dementia (Oddo et al., 2003; Mastrangelo and Bowers, 2008). At 3 months, labelling of both APP and A$_\beta$ becomes increasingly more intense and widespread and occurs within many regions of the HC, amygdala, and layers II and II of the EC as well as neocortex. This intensity and level of intracellular A$_\beta$ continues to rise at 6 months and continues until 12 months, when evidence of tau pathology becomes apparent. Although hyperphosphorylated tau is evident, particularly in the CA1/Subiculum regions of the hippocampal formation (HF), paired-helical filaments only become evident at 18 months.

It is evident that this AD-like pathological presentation is found to originate specifically on within the border of CA1 and subiculum and progresses to affect other hippocampal and non-hippocampal regions with age. In support of this, the study
reports that intracellular Aβ is followed by the formation of Aβ plaques, and again is first apparent in this region of the HF. The study also reports that intracellular Aβ pathology is found not only within the cell body, but also within the dendritic and terminal regions of the neuron. As a result, this could be important to take into account when assessing the electrophysiological properties of the neurons within the affected regions.

In support of theories suggesting that Aβ pathology affects tau pathology and that Aβ development is independent of tau, here they report that tau pathology is found to co-localise with Aβ positive neurons (Mastrangelo and Bowers, 2008; Takahashi et al., 2010). However, there are limitations associated with this study. Whilst this is the first and only comprehensive study on the pathological phenotype of this model, the authors conclusions are based solely on qualitative measurements. As such, there is a clear need for a quantitative study to be conducted in order to reliably correlate the pathological, behavioural and electrophysiological phenotype of these mice.

Cognitive and behavioural phenotype

The cognitive and behavioural phenotype of the 3xTgAD mouse has been extensively studied through the use of a battery of tests, including; anxiety, locomotion, object recognition, fear conditioning, spatial and episodic-like memory (Billings et al., 2005; Clinton et al., 2007; Gulinello et al., 2009; Pietropaolo et al., 2008; Davis et al., 2013b,a). Again, many studies have confirmed that these mice are cognitively intact at birth and develop these impairments due to the onset and progression of AD-like pathology, with cognitive impairments becoming apparent at 4-6 months (Billings et al., 2005; Davis et al., 2013b,a; Clinton et al., 2007). One limitation associated with many behavioural paradigms is that they aim to test individual aspects of cognition. Human cognitive and memory impairments are complex and involve a number of aspects simultaneously, and therefore cognitive tests that focus on single aspects are likely to be to simplistic. In response to this, a test for episodic-like memory was designed and implemented in the double APP/PS1 AD mouse (Dere et al., 2005). This test relies on the subject’s ability to integrate various individual components of tasks in order to form and recollect event-based memories, an important cognitive function that is lost in human AD. This ‘what-where-when’ test can be solved by reference to a previously encoded event, satisfying Tulving’s definition for episodic memory (Tulving, 1972); however, it is also open to a non-episodic solution via recognition of relative familiarity with the test objects (Easton et al., 2012). Recent work utilised a different paradigm that is not open to a non-episodic solution (Norman and Eacott, 2004) and assessed the performance of 3xTgAD mice in the individual
1.3. The Hippocampus

In humans the HC is a bilateral sea horse shaped structure located deep within the medial temporal lobes. The HF forms part of the system involved in the formation, storage and retrieval of memories where it plays an important role specifically in the consolidation and retrieval processes (Penfield and Milner 1958; Squire and Zola-Morgan 1991; Squire 2004). Importantly, the HC has an unique ability to integrate memories associating object, place and time; forming episodic memories (Tulving 1972; Dere et al. 2006; Davis et al. 2013b,a). This sub-type of memory results in the ability for us to re-visit past event-based memories and make decisions and predictions for the future based on these. Damage to the HF through surgical components and a combined version of this task (Davis et al. 2013b,a). In this study the authors show that these mice show episodic-like memory deficits at 6 months, however, they also show decreased task performance when compared to age-matched control mice as early as 3 months. They also report that mice are able to perform to ‘normal’ levels in the individual components, but are impaired when bringing them together to form associations. Interestingly, whilst mice at this age are able to perform the task, albeit to a decreased level, this provides evidence of subtle changes in cognitive performance at 3 months that may represent an MCI-AD converter phenotype (Davis et al. 2013b,a).

Whilst there is no known neurological change currently associated with changes in memory performance in AD, recent work has reported possible changes in the spatial resolution of hippocampal maps that may help explain the spatial memory deficits in AD (Cacucci et al. 2008; Cheng and Ji 2013). Cacucci and colleagues show evidence of a reduction in the spatial resolution and a concurrent increase in the relative size of the response in place cells in the aged Tg2576 mouse (Cacucci et al. 2008). Cheng and Ji, however, show a loss in the ability of place cells to respond to external cues in the environment in a taupatology mouse model (Cheng and Ji 2013). There has been little other research investigating if and how there are changes in brain regions responsible for the spatial mapping of the external world in AD, however, this could provide one potential mechanism underlying spatial memory dysfunction.

The cognitive and behavioural assessment of the 3xTgAD mouse has led to further support that cognitive and functional changes are evident prior to overt plaque and tangle pathology. It is now believed that these cognitive changes are correlated with the development of intracellular oligomeric forms of Aβ rather than the later pathological forms (Billings et al. 2005; Oddo et al. 2003).

1.3 The Hippocampus

In humans the HC is a bilateral sea horse shaped structure located deep within the medial temporal lobes. The HF forms part of the system involved in the formation, storage and retrieval of memories where it plays an important role specifically in the consolidation and retrieval processes (Penfield and Milner 1958; Squire and Zola-Morgan 1991; Squire 2004). Importantly, the HC has an unique ability to integrate memories associating object, place and time; forming episodic memories (Tulving 1972; Dere et al. 2006; Davis et al. 2013b,a). This sub-type of memory results in the ability for us to re-visit past event-based memories and make decisions and predictions for the future based on these. Damage to the HF through surgical
lesions, accidental damage or disease (such as in AD) results in the loss of function of this important memory system, with patients/subjects losing the ability to form and retrieve memories to differing degrees (Milner, 1959; Selkoe, 2011; Penfield and Milner, 1958).

The HC forms part of a wider hippocampal-cortical processing system that is conserved across all mammalian species (Buckner, 2010). It is located in such a position to receive multi-modal input from the cortex, which it processes and returns the information to the site of cortical origin. Therefore, the HC is traditionally seen as the interface between the acquisition and formation of memories, and the storage of these in the neocortex (Scoville and Milner, 1957; Frankland and Bontempi, 2005).

The HF is a collective term used to describe a number of associated sub-regions including; perirhinal cortex, parahippocampus (EC, parasubiculum, presubiculum), dentate gyrus (DG), subiculum and the HC (consisting of fields CA3, CA2 and CA1). The HF was initially viewed as a serial processing loop, with information flowing in a predominately unidirectional manner within a trisynaptic processing loop, dominated by feed-forward excitatory connectivity (Andersen et al., 1971). In contrast to this initial theory, the processing pathway within the HF is now seen as a much more complex system, consisting of a variety of serial and parallel inputs and outputs (see Figure 1.3).

The EC acts as the interface between the neocortex and the HC. Here it receives a variety of inputs from cortical and sub-cortical regions, and relays this information to the HC via layers II/III of the EC. These hippocampal inputs, arising from the EC, enter the HF either directly or indirectly in the DG, CA3 (from EC layers II), CA1 and subiculum (via layers III). The information is processed through the system and is eventually relayed back to deeper layers IV/V of the EC via CA1 and subiculum (Amaral and Witter, 1989; Andersen et al., 1966; Witter and Amaral, 1991; Andersen et al., 1973; Gigg, 2006).

In detail, input arriving via layer II of the EC (known as the perforant path) terminates in and forms synapses with the pyramidal cells of DG and CA3 via mossy fibres, which in turn, via the Schaffer collaterals, forms synapses in the CA1 pyramidal cell layer and CA1 s.r. Synaptic activity is then relayed back to the EC directly from CA1, via further processing through the monosynaptic connections projecting from CA1 to the subiculum and then back to EC (Amaral and Witter, 1989; Andersen et al., 1966; Witter and Amaral, 1991; Andersen et al., 1973; Gigg, 2006). However, information can be processed through a slightly different and more direct pathway. Inputs arriving from layer III of the EC (known as the temporoammonic pathway (TA)) project directly to CA1 and subiculum, which in turn project as hippocampal...
outputs to the deeper layers (IV and V) of the EC. Information can then either be sent to the cortex for further processing or storage, or can be sent back to the EC superficial layers (II/III) and back into the HC. This process, known as hippocampal re-entrance (see Figure 1.3), is important for comparison and refining hippocampal connections (Kloosterman et al., 2003, 2004).

Other regions of the HF, such as the perirhinal and postrhinal cortex feed contextual information into the HC aiding the formation of associative memories. For example, whilst the perirhinal cortex has been shown to be involved in the processing of object information, the postrhinal cortex is known to be involved in spatial memory (Naber et al., 2000). Importantly, regions within the HF are known to have the ability to modify and strengthen synapses and, therefore, express activity-dependant synaptic plasticity, a function that is vital in making new memories (Nabavi et al., 2014; Bliss and Lomo, 1973; Raymond, 2007; Lombroso and Ogren, 2009; Davis et al., 2014; Gigg, 2006). Synaptic plasticity is thought to provide a cellular mechanism for memory formation and refinement of these memories. As such, synaptic plasticity within the HF is key to its function.

Memories can be split into two distinct forms; procedural and declarative. Procedural memories are typically those that require motor and knowing how to perform actions, whilst declarative memories are those that store events and facts (Eichenbaum, 2000; Dere et al., 2006). Early studies indicated that different regions of the brain create these two forms, with the HC found to be vital for the formation of declarative memory (Scoville and Milner, 1957). As discussed earlier, the HF plays a vital role in the formation and retrieval of memories. As such, it must therefore have the ability to a) initially form novel memory traces, and b) facilitate the long-term storage of these memories in other brain regions. Studies suggest that the HC is important for acquiring new declarative memories and then later, during a consolidation phase, sends these for long-term storage within the neocortex (McClelland et al., 1995). As such, the HC is thought to be in one of two states at any one time, offline and online. The acquisition of memories is thought to take place predominately during the ‘online’ period, whilst consolidation of these memories is facilitated by periods of sleep, during the ‘offline’ phase. In order to strengthen their long-term storage, memories are thought to be ‘replayed/revisited’ within the neocortical network and then associated with other memories (Pavlides and Winson, 1989; Wilson and McNaughton, 1994). Further evidence of this stems from surgical intervention and disease, where lesions to the HC are found to disrupt recent memories, whilst remote memories remain intact (Alvarez and Squire, 1994; Estmacott and Moscovitch, 2002; Zola-Morgan and Squire, 1990; Anagnostaras et al., 1999; Winocur, 1990).
Figure 1.3. Hippocampal connectivity. (A and B) Schematic illustrating the glutamatergic connections throughout the hippocampal formation. Information first enters the HC via the superficial layers (II and III) of the EC. Inputs arising from layer II form the perforant pathway and project to the granular layer of the DG. Cells from the DG send feed-forward excitatory projections via mossy fibres to CA3, which in turn project to CA1 via the Schaffer collaterals. Inputs arising from EC layer III form the temporo-ammonic pathway, and synapse directly with CA1 and subicular neurons. CA1 pyramidal cells form monosynaptic connections with subiculum. Information returns to EC layers V/VI (and weakly to layer III) via CA1 and/or subiculum. Processed information is then either sent to the neocortex, or back to superficial regions of the EC and back into the HC (‘re-entrant’ loop). Recent work has shown evidence of subicular inputs to both CA1 and C3 (indicated by green arrows), however, the specific anatomical positions of these inputs has not been described (Sun et al. 2014; Jackson et al. 2014).
1.3.1 Field CA1 and subiculum

As discussed earlier, the HF consists a number of sub-regions, each of which plays an important role in the formation and retrieval of a variety of memories. Cornu Ammonis field CA1 and subiculum are seen as the final regions of this system, prior to output of information to other cortical regions (Witter and Amaral, 1991; Amaral and Witter, 1989). Therefore it is important to focus on understanding the function and mechanisms of these two regions of the development memory. The CA1 is a structurally simple laminated region, and is one of the most extensively studied regions of the HC and is involved in the generation of internal spatial maps and is vital for the function of spatial and working memory (O’Keefe and Dostrovsky, 1971; O’Keefe and Nadel, 1978; Bures et al., 1997; Smith and Mizumori, 2006; Muller et al., 1987).

The subiculum is positioned between the hippocampus proper (fields CA3, CA2 and CA1) and the EC in a location that enables it to act as a pivotal structure prior to hippocampal output. The subiculum receives direct input from EC and CA1, with CA1 inputs arriving in a topographical manner. These inputs are arranged such that proximal CA1 (part closest to CA3) forms monosynaptic connections with distal subiculum (part furthest from CA3), distal CA1 forms connections with proximal subiculum, and inputs arising from medial portions of CA1 provide inputs to medial subiculum (O’Mara, 2005; Gigg, 2006; Witter and Amaral, 1991). Although this topographical organisation suggests that specific regions of CA1 solely activate discrete portions of subiculum, studies now show that recipient ‘columns’ in subiculum share strong lateral axonal communication and, therefore, information arriving at any one part of subiculum is likely to be ‘shared’ across other parts (Witter, 1993; Harris and Stewart, 2001).

Whilst the subiculum is strategically placed as the final region prior to hippocampal output to the EC/neocortex, there is a clear paucity of research into its physiological function, leaving it a relatively poorly understood region of this important system. In addition, recent work has implicated the subiculum as a possible input structure of the HC. Tracer studies have shown evidence of large excitatory and inhibitory connections arising within subiculum that project directly to CA1 (Sun et al., 2014). Further research has also shown that oscillatory activity can be generated independently within the subiculum, and that this activity regulates that found within CA3 (Jackson et al., 2014).

The subiculum can be split into two sections, the dorsal and ventral system, each having a defined and clear physiological role, with the dorsal subiculum involved in memory formation whilst the ventral region is involved in regulating the
hypothalamic-pituitary-adrenal (HPA) axis (O’Mara, 2005; Bannerman et al., 2004). The ventral subiculum provides an inhibitory ‘tone’ to the HPA and is extensively connected with the amygdala, Raphe nucleus and as result plays a key role in the control of emotion. As such, dysfunction of the ventral subiculum can facilitate neuropsychiatric conditions such as depression and schizophrenia (O’Mara, 2005; Canteras and Swanson 1992; Behr et al., 2000).

The dorsal subiculum is traditionally seen as the output region of the dorsal HC where it receives and integrates inputs from an array of regions; including, EC and HC. Importantly, its role in the processing of memory-relevant information is supported by the presence of spatially correlated neuronal activity. Unlike the place cells (PC) found within CA1, place modulated cells within the subiculum tend to contain multiple peaks of activity across the environment and therefore result in poor spatial resolution compared to CA1 (Sharp and Green, 1994; O’Mara, 2005; O’Keefe and Dostrovsky, 1971; Smith and Mizumori, 2006). Research has shown that spatially sensitive neurons in the subiculum are less spatially coherent when compared to CA1 cells. Therefore, they suggest that this could arise if a number of CA1 place cells were to converge onto individual cells within subiculum (Sharp and Green, 1994).

Further evidence in support of the similar yet different role of the subiculum in spatial navigation stems from the key difference between the robustness/remapping of cells in different/novel environments. CA1 PCs are found to remap in very different environments, and therefore CA1 place fields are dynamic, however subicular maps remain static in different environments. As such, subicular neurons are found to fire in robust patterns between different environments. In addition, whilst CA1 contains one type of spatial cell, the subiculum contains an array of units, each designed to build a robust map of the external world, mapping different aspects of properties of the environment. These cells currently include; boundary vector cells and border cells (Solstad et al., 2008; Lever et al., 2009; Stewart et al., 2014).

Interestingly, spatially responsive cells within the subiculum are able to generate internal maps of the external world independently of direct input from any other region of the HC. This provides evidence to support the independent function of the subiculum, therefore allowing for the subiculum to generate a universal map across all environments which are in turn modified and refined by other hippocampal cells (O’Mara et al., 2009). In addition, recent work has found spatially sensitive boundary vector cells within the subiculum, and the firing properties of these are likely to help generate the CA1 place cell firing (Lever et al., 2009; Stewart et al., 2014). This, therefore, challenges the view that the subiculum merely acts as a hippocampal output region, and that it might in fact provide an important input of spatial information. In support of the relatively new view of the subiculum, other research has demonstrated that subicular cells can become activated prior to those
in CA1, with CA1 cells becoming activated during later stages of memory tasks (Deadwyler and Hampson 2004; Cappaert et al. 2007). Work by Deadwyler and Hampson (2004) suggests that the subiculum signal is generated prior to that in CA1 and is able to function as a temporary memory buffer before passing information back to the HC for further processing.

### 1.3.2 Synaptic plasticity

Both short- and long-term synaptic plasticity have long been proposed to act as the biological mechanism underlying memory formation, resulting in activity-dependant changes in synaptic efficacy (Liu et al. 2008). The first experiment to demonstrate long-lasting alterations in synaptic strength to a high-frequency stimulus (HFS) within the HC was conducted in 1973 by Bliss and Lomo. This resulted in long-term potentiation (LTP), resulting in an increase in the synaptic strength of the perforant pathway to DG (Bliss and Lomo 1973). LTP is described as the long-term enhancement in the strength of synaptic transmission between two or more neurons, and has been widely demonstrated throughout all regions of the HC, including the subiculum (Behr et al. 1998; Commins et al. 1998; Bliss and Lomo 1973; Craig and Commins 2006; Gureviciene et al. 2004).

Both, short- and long term plasticity result in the increased synaptic efficiency between two or more neurons, yet they have separate mechanisms of induction. Application of a high frequency stimuls (HFS) to presynaptic fibres in a typical glutamatergic synaptic connection (e.g., CA3→CA1) results in sustained activation of the postsynaptic dendrites. Summation of these inputs results in sustained activation of AMPA receptors on the postsynaptic neuron. This sustained postsynaptic depolarization removes the magnesium block of NMDA receptors, thereby allowing calcium entry and increased intracellular calcium levels postsynaptically. This leads to activation of various protein kinases and trafficking of AMPA receptors to the dendrites followed by activation of metabotropic glutamate receptors. This additive affect results in up-regulation of the transcription of genes, leading to a long-term (hours to months) enhancement of synaptic connectivity within activated synapses (Bliss and Collingridge 1993; Bliss and Gardner-Medwin 1971; Bliss and Lomo 1973; Lisman 2009).

Whilst LTP is typically induced in response to sustained application of a HFS, short-term plasticity is induced in response to paired-pulse stimulation, and lasts only as long as the stimulus event and is termed ‘paired-pulse facilitation’ (PPF). Application of pairs of pulses to the presynaptic neuron typically results in the response to the second stimuli being facilitated when compared to that produced in
response to the first and typically lasts for a relatively short period (ms-s) \cite{Zucker1989, Commins1998}. The mechanism of induction of PPF is presynaptic with a key emphasis on the residual calcium hypothesis \cite{Katz1968, Zucker2002}. However, it is likely that other mechanisms play an important role; for example, activation of protein kinases known to modulate the activity of presynaptic phosphoproteins have also been shown to be involved in the process of PPF induction \cite{Rosahl1993, Rosahl1995, Citri2008}.

In the residual calcium hypothesis, stimulation of the presynaptic neuron results in the generation of an action potential and activation of voltage gated calcium channels at the axon terminal, leading to calcium influx and neurotransmitter release. If a second stimulus then arrives at the presynaptic neuron within a short period following the first, prior to degradation of the first presynaptic response, the ‘residual’ influx of calcium in response to the first stimuli now combines with that due to the second, resulting in an additive effect and a greater release of neurotransmitter. This increased release leads to increased activation of the postsynaptic neuron, whilst the intensity of the stimulation remained constant.

Short-term increases in synaptic efficiency can also be induced during trains of repetitive stimuli presented at high frequencies (10-200Hz), lasting for a period of minutes \cite{Zucker2002}. This type of facilitation is known as post-tetanic potentiation (PTP) and is induced due to a build-up of intracellular calcium in the presynaptic terminal, again due to the additive effects of previous stimuli. Importantly, whilst PPF and PTP tend to lead to increases in synaptic strength, they can also result in depression of the response. This occurs when the presynaptic neuron already has a high release probability, quickly resulting in vesicle depletion with each successive stimulus.

Neurons that have a low release probability will have a greater propensity to facilitate in response to repetitive stimuli, such as those found in bursting neurons, and therefore act as high pass filters. However, neurons with a high release probability are much less likely to facilitate in response to bursting stimuli, and therefore act as low-pass filters, favouring transmission of information during low-frequency stimuli \cite{Abbott2004}. These low and high release probabilities are therefore likely to be directly involved in the induction of LTP. The mechanism for LTP induction has, for many years, been an area of great debate. Are the mechanisms purely of a pre-synaptic or a post-synaptic nature, or are they due to combination of both?. Research has provided evidence of both mechanisms \cite{Enoki2009, Ahmed2009, Kerchner2008}. However, it is possible that those neurons that have low release probabilities partake by increasing vesicle release, and post-synaptic mechanisms will account for induction due to neurons
that have high release probability prior to LTP induction.

### 1.3.3 Assessment of neuronal function of Alzheimer’s disease mice

Whilst the mechanisms and physiological relevance of synaptic plasticity has been widely studied in the HC, there has been limited work assessing this functional neuronal connectivity through electrophysiological recordings in AD models, particularly *in vivo*. Within these few studies, conclusions and results often conflict, which is most likely due to the small volume of research, variation in mouse models, preparation (*in vivo* vs *in vitro*) and regions assessed at different stages of disease progression.

To my knowledge, there have only been two studies assessing synaptic connectivity in the 3xTgAD mouse. The first of these focused on the synaptic integrity of the Schaffer collaterals through stimulation of CA3 pyramidal cells. Here they used a low frequency train of stimuli at increasing intensities in order to test for changes in synaptic connectivity. They concluded that at 2 months, CA3→CA1 connectivity was intact in 3xTgAD mice, but decreased by 6 months. In addition, at 6 months, 3xTgAD mice also displayed evidence of deficits in LTP induction following application of a HFS [Oddo et al., 2003]. More recently, research assessed the synaptic function between the subiculum and re-entrance of hippocampal activity into the DG and CA1 [Davis et al., 2014]. Again, using a low frequency train of stimuli, synaptic inputs following subicular stimulation, remained intact in the DG and CA1 in both young (4-6m) and old (17-18m) mice. Interestingly, whilst the synaptic connections remained intact, deficits in the control of synaptic activity during PPS became evident. At 4-6 months, 3xTgAD field excitatory post-synaptic potential (fEPSPs) in both DG and CA1 were facilitated to those in age-matched control mice, whilst the latency of the response remained comparable between genotypes. Paired-pulse responses were found to decrease as a function of age in both genotypes, however, where responses were depressed in response to the second pulse in control mice, 3xTgAD responses facilitated at short intervals. As such, the authors provide strong evidence in support of synaptic hyperexcitability in dorsal EC-DG-CA1 circuitry, particularly in young mice. In support of this, the authors also show that young 3xTgAD mice display evidence of greater probability for reverberation of synaptic activity through the hippocampal circuitry during stimulus trains [Davis et al., 2014] (see Figure 1.4). This functional change in the hippocampal circuitry correlates well with the onset of cognitive changes at comparatively early age in an episodic-like memory task [Davis et al., 2013b,a]. Whilst this research focused on evoked field responses, other research has documented changes in the spontaneous oscillatory pattern of LFPs, indicated by an increase in epileptiform activity in AD mice, which is again in support of a hyperexcitable phenotype [Palop et al., 2007]. These changes...
are thought to be related to alterations in the excitatory and inhibitory regulation of neuronal synapses. Specifically, the accumulation of intracellular Aβ is known to cause a decrease in presynaptic glutamatergic transmission, however, recent work in animal models and human AD has shown increased incidence of neuronal hyperexcitability with Aβ accumulation \cite{Palop2007, Kamenetz2003, Davis2014}. Therefore, it is possible that Aβ accumulation has a complex interaction with neuronal populations, possibly by co-existence of decreased glutamatergic transmission and an increase in excitability due to Aβ-induced suppression of inhibitory neurons \cite{Palop2007}.

Whilst this study suggests that the basal synaptic connectivity in the 3xTgAD mouse remains intact with age and disease progression, other studies conducted in the APP\textsubscript{SWE} mouse indicates that basal connectivity is down regulated \cite{Fitzjohn2001, Jacobsen2006}. In support of the changes associated with disease found in the 2003 study by Oddo and colleagues, other research shows changes in LTP induction and maintenance in different models, including the APP+Tau, PS1, APP\textsubscript{SW,E}, APP\textsubscript{SW,E}+PS1\textsubscript{A246E} mouse \cite{Chong2011, Wang2009, Chapman1999, Jacobsen2006, Gengler2010, Gureviciene2004}. However, it is important to take into account that the majority of this research has been conducted in the reduced slice preparation, each of which would significantly vary in the exact physiological parameters and remaining connectivity. Clearly, this therefore only provides a small window into the potential changes associated with disease and supports the requirement of further investigation through \textit{in vivo} research.

Although we do not currently fully understand the mechanism behind these functional changes, it is widely accepted that alterations in neuronal function correlate with pathology. Early synaptic deficits in AD mice are thought to be associated with the accumulation of Aβ oligomers which, at pathological concentrations, are known to alter synaptic plasticity \cite{Selkoe2008}. Whilst not showing a direct link to intracellular Aβ oligomers, intracerebroventricular injections of Aβ\textsubscript{1−42} oligomers into otherwise ‘healthy’ rats results in cognitive impairments and changes in synaptic plasticity; supporting the detrimental role of an oligomeric form of Aβ \cite{Wu2008, Shankar2007, Shankar2008, Li2009}. This increase of intracellular Aβ is also found to correlate with an age-related loss of nicotinic acetylcholine receptors at 6 months and has been suggested to influence the loss of LTP induction in the 3xTgAD mouse \cite{Oddo2003}. Indeed, addition of Aβ\textsubscript{1−42} oligomers to cell cultures result in changes in cell activity and cell death \cite{Shankar2007, Shankar2008, Li2009}. In addition to the underlying pathology, the introduction of the transgenes themselves is also known to affect the physiological regulation of synaptic function, particularly through the introduction of the PS1 gene \cite{Palop2007}. 
1.3. The Hippocampus

Figure 1.4. Electrophysiological recordings in the 3xTgAD mouse. (A) and (B) CA1 and DG synaptic responses in response to subiculum single-pulse stimulation in young (4-6 months) and old (17-18 months) 3xTgAD and control mice. These results show that there were no significant genotype difference between and within young and old mice and, therefore, they have intact basic functional synaptic connectivity between EC and CA1/DG. In contrast, Oddo et al. (2003) showed evidence of basic synaptic dysfunction in 6 month old 3xTgAD mice in the CA3-CA1 Schaffer collateral pathway (C-left). They also demonstrated deficits in LTP induction in these mice (C-right). Note that all stimulation was conducted at amplitudes that elicited half-maximal responses in both experiments and that results in (A) and (B) are from in-vivo recordings and (C) were from in-vitro recordings. (Figures adapted from Davis et al. (2014) and Oddo et al. (2003).
1.3. The Hippocampus

Oddo et al., 2003).

Studies show that the PS gene results in changes in cholinergic function, abnormal NMDA currents and alterations in the muscarinic modulation of LTP (Wang et al., 2009). Additionally, PS is known to be involved in calcium homeostasis, resulting in enhanced calcium release from the endoplasmic reticulum (Wang et al., 2009). This increase in calcium release is thought to impair LTP induction through suppression of calcium-dependant NMDA receptors due to hyperpolarisation of pyramidal cells (Wang et al., 2009). However, whilst studies implicate the role of the PS1 gene in calcium dysregulation, research conducted by Oddo and colleagues argues that the introduction and co-expression of the APP_{SWE} transgene negates the PS1 induced effect on calcium regulation (Oddo et al., 2003). However, this is still under debate, with other studies suggesting that this is not the case. Caffeine-induced calcium release is upregulated in the 3xTgAD mouse, with increased expression of ryanodine receptors (which are important in calcium-induced calcium release) (Smith et al., 2005). Other research also shows that changes in calcium homeostasis are still present, with increased levels of resting calcium concentrations found within cortical neurons (Lopez et al., 2008).

Whilst it is widely accepted that there is likely to be an alteration in calcium homeostasis, other studies suggest additional changes in cholinergic modulation of the glutamatergic system (Goto et al., 2008). Application of cholinesterase inhibitors results in a significant reduction in the fEPSP of CA1 responses in 5 month old APP/PS1 mice when compared to age-matched controls. This effect was rescued following application of atropine (a muscarinic antagonist). Therefore, the authors suggest that there are muscarinic impairments in AD mice, leading to inhibition of glutamatergic transmission or an increase in the release of GABA (Goto et al., 2008).

With the limited number of studies conducted to date that assess neuronal function within the HF in AD, and the fragmented results due to the differences in method and preparations, it is evident that further investigation is required. With only one in vivo study conducted in the 3xTgAD mouse (Davis et al., 2014) and only a few reported in other mouse models (Gureviciene et al., 2004; Gengler et al., 2010; Witton et al., 2014; Cheng and Ji, 2013; Scott et al., 2012), it is vital that we further our understanding through investigation of the full hippocampal circuitry in a fully intact in vivo preparation.

So far we have discussed the effect of AD and AD pathology of the activity on individual/groups of neurons through the assessment of evoked responses. However, it is also important to investigate the effect of AD on neuronal populations. Therefore, the next sections within the chapter will focus on the role of network oscillations
and how these are affected in AD.

### 1.4 Neuronal oscillations and their role in memory

Oscillations in local field potentials (LFPs) are the result of the synchronous firing of populations of neurons within subcortical and cortical regions. This rhythmic pattern of firing activity gives rise to the generation of state-dependant large-scale changes in EEG voltage. This oscillatory activity represents the changes in potential due to a function of the strength of the activity, distance and coherence with other currents. As such, these LFPs are generated from a complex integration of local and distal current ranging from fast inputs via action potentials to slow glial fluctuations (Buzsáki et al., 2012). Typically, this oscillatory activity can be recorded outside the skull (EEG), from the brain surface (ECoG) or directly through the insertion of recording electrodes directly into the region of interest.

Fast and slow synaptic activity results in the combination of dendritic activation and neuronal spiking activity. This pattern of activity can be measured as a potential difference across the cell membrane and can be recorded both intra and extracellularly (Buzsáki, 2004). Dendritic activation leads to a net influx of positive change, resulting in the immediately surrounding extracellular space becoming negatively charged due to the relative loss of positive ions. This is typically referred to as a current sink. However, for a circuit to be established, this relative increase in intracellular current must be returned back to the source; the extracellular space, leading to a current source. This sink-source coupling leads to a dipole being established. In a highly laminated structure, such as that of the HC, this leads to a large summation of current sinks and sources, allowing for recording of large-scale LFPs.

The synchronous pattern of activity recorded by these methods is thought to underlie the mechanism of cellular communication, providing a window of opportunity for global communication between populations of temporally coherent circuits within the brain. Since this rhythmic activity is essentially generated as a summation of local and distal inputs, it is conceivable that the frequencies at which these rhythms can be generated can vary. Indeed, neuronal oscillations have been recorded and separated into different frequency bands; e.g., slow-wave, delta, theta, alpha, beta and gamma frequencies. Whilst it is generally accepted that these bands are present in all animals, the frequency bands over which you find these physiologically important oscillations varies depending on species and specific experimental preparation (e.g. *in vitro* vs *in vivo*; anaesthetised vs awake).
1.4. Neuronal oscillations and their role in memory

1.4.1 Types of oscillations

EEG/LFP rhythms have been found to be heavily involved in the processing of information for functions, such as memory and motor output. Interestingly, different EEG/LFP frequency bands are often found simultaneously and such a combination of frequencies are essential for the correct processing of neuronal information. For example, both theta and gamma rhythms are frequently observed simultaneously, with both rhythms involved in the process of memory acquisition (Lisman and Idiart, 1995; Lisman and Buzsáki, 2008). Some particularly relevant EEG/LFP bands for the present project are described further below.

Slow-wave and delta oscillations

Slow-wave (<1Hz) and delta (1-4Hz) oscillations result in large amplitude rhythmic fluctuations in the LFP and tend to dominate the EEG signal during periods of rest and deep (non-REM) sleep. During these periods, the EEG signal is complex and consists of a number of other physiologically important rhythms that occur during periods of delta activity. The latter include spindles and slow-wave complexes (140-200Hz) which co-occur during periods of rest and deep sleep (Buzsáki et al., 1983; Chrobak and Buzsáki, 1996; Csicsvari et al., 1999).

Delta rhythms are generated from thalamic input to the HC and cortex, resulting from a decrease in cholinergic and amnergic inputs. These thalamic neurons tend to fire in bursts, patterns that coincide with the frequency of delta oscillations (Amzica et al., 1992; Steriade et al., 1991). In addition to the delta component, slow-wave oscillation is also intrinsic to deep sleep (Clement et al., 2008). This very slow component is present during all periods of delta activity and is thought to bind the state together (Steriade et al., 1993; Steriade, 2006). Delta and slow-wave rhythms are also entrained within the HC, following cortical input to the EC, via inputs from the TA pathway to CA1. This results in a cortico-hippocampal coupling during this state, with the cortical activity preceding hippocampal activity by tens of milliseconds (Taxidis et al., 2013). Activity of hippocampal pyramidal cells and interneurons are paced by the delta/slow-wave activity, resulting in neuronal coherence between the HC and the cortex, therefore, potentially facilitating the transfer of information during this window of activity.

Whilst delta and slow-wave frequencies are generated and controlled by hippocampal inputs, slow-wave complexes are controlled by intra-hippocampal mechanisms, and are found throughout the HF, including subiculum, parasubiculum and deep layers of the EC (Buzsáki et al., 1983; Csicsvari et al., 1999; Chrobak and
These ultra-fast, slow-wave complexes are due to the depolarisation of CA1 pyramidal cells resulting from CA3-Schaffer collateral recruitment. Therefore they are generated due a combination of inputs arising from both CA3 and CA1 as a result of the synchronous somatic inhibitory inputs (Buzsáki 1986). 

Theta rhythms

Theta rhythms generally oscillate at frequencies between 3-8Hz and are typically associated with exploratory activity, acquisition and processing of memory and REM-sleep (Givens and Olton 1990; Berry and Seager 2001; McNaughton et al., 2006).

Theta generation is dependant on inputs from pacemaker regions. These have both intrinsic and extrinsic origins with CA3, subiculum and layers II and III of the EC providing intrinsic inputs, and the medial septum providing the extrinsic input (Vertes and Kocsis 1997; Jackson et al., 2014; Hangya et al., 2009). Strong rhythmic inputs arising from the medial septum are provided by a combination of GABAergic, cholinergic and glutamatergic neurons, with a sub-population of GABAergic neurons acting as a pacemaker (Hangya et al., 2009). The rhythmically firing neuronal activity within the medial septum precedes that of the HC, therefore, is generated prior to hippocampal rhythms. The GABAergic inhibitory neurons form connections with and inhibit local inhibitory interneurons, leading to the disinhibition of hippocampal pyramidal cells (Hangya et al., 2009; Toth et al., 1993; Vertes and Kocsis 1997).

The fluctuation of neuronal activity within the medial septum and HC results in an oscillation of current between the dendritic and somatic region of the cells. In addition to the GABAergic projections, inputs arising from cholinergic neurons play a key role in the modulation of the underlying theta rhythm. Whilst their firing dynamics are much slower than the theta frequency and therefore do not act as direct pacemakers, they are known to have a modulatory effect such as controlling theta power (Apartis et al., 1998; Simon et al., 2006; Yoder and Pang, 2005).

In addition to pacemaker inputs arising from the medial septum, inputs arising either directly or indirectly from the EC are also implicated in the production of the theta sink/source dipole in both the DG and CA1 (Hasselmo, 2005). As a result of the simultaneous inputs arising from the medial septum and EC, a theta dipole is generated. Note that the strongest input is generated in the CA1 distal apical dendrites as a result of the influence of EC fibres. Due to the differences in the strength of the inputs, as you progress towards the soma and basal dendritic regions, the power of CA1 theta decreases and a 180-degree phase shift is observed.
Gamma rhythms

Gamma rhythms occupy a broad frequency range (30-100Hz) with an additional ultra-fast gamma rhythm typically observed during periods of sleep (>100Hz) (Draguhn et al., 2000). Gamma is generated intrinsically through activity of fast-spiking inhibitory basket cell interneurons and concurrent activity with excitatory neurons, modulated by GABAa receptors (Whittington et al., 2000; Hasenstaub et al., 2005; Economo and White, 2012). Interestingly, gamma rhythms are commonly found to co-occur during periods of theta, with gamma power modulated by theta phase (Axmacher et al., 2006; Lisman and Buzsáki, 2008).

The generation of network oscillations within the gamma frequency band depends on the synchronous activity of fast-spiking parvalbumin (PV) inhibitory interneurons. The role of these neurons and their subsequent network oscillations are important for normal control of neuronal excitability with dysfunction leading to cognitive and psychological impairments, such as those found in schizophrenia and autism (Fazzari et al., 2010; Sohal et al., 2009). The function of PV interneurons are vital for controlling and fine-tuning of network oscillations due to their modulatory effect on excitatory pyramidal cells (Freund and Katona, 2007). Parvalbumin cells account for around 40% of all inhibitory interneurons within the HC and provide recurrent somatic and perisomatic inhibition to pyramidal cells. This recurrent excitatory-inhibitory coupling leads to tight control of spiking activity, resulting in the generation of high frequency network oscillations between 30-100Hz. The frequency of gamma oscillations is directly related to the firing rate of PV interneurons (Cardin et al., 2009).

1.4.2 What is sleep and what function does it serve?

Prior to discussing the role of various network oscillations in memory, it is important to briefly discuss the role and function of sleep. As mentioned earlier, there are ranges of network rhythms that are detectable in the brain, however, many of these are state dependent, with many EEG bands becoming more prominent during periods of sleep.

Sleep can be described as a reversible state of reduced responsiveness associated with inactivity and loss of consciousness (Rasch and Born, 2013). During sleep, neuronal oscillations cycle between slow-wave, non-rapid eye movement (non-REM) and theta dominant REM sleep. Non-REM sleep is dominated by periods of high amplitude slow-wave, delta, spindles and slow-wave complexes, whilst REM sleep is dominated by smaller amplitude theta and gamma activity. Whilst sleep is a function
in which we all participate, its function its not fully understood; however, the possible roles of sleep include restoration and cell repair, thermo- and metabolic regulation, detoxification and removal of free radicals, facilitation of memory consolidation and the pruning synaptic connections \cite{Berger1995, Bach2011, Eckel-Mahan2013, Inoue1995, Scharf2008, Tononi2006, Csicsvari1998, Buzsaki1998}.

### 1.4.3 The physiological relevance of network oscillations

Communication between neurons within ensembles locally and across brain regions is supported by action potentials in the form of individual spikes or spike bursts \cite{Kepecs2003}. There are currently two theories of how information can be transmitted between brain regions. The first relies on the physical connectivity between two or more neurons, whilst others believe that synaptic information can be relayed between different parts of the brain through neuronal coherence, during which a number of regions will be temporally coherent and therefore this acts as a ‘window of opportunity’ to transmit information \cite{Fries2005, Axmacher2006}. In order for regions to express neuronal coherence, they must rely on rhythmic fluctuations in large populations of neurons. This results in the generation of large network oscillations and, if other regions are temporally and spatially coherent, then it is likely that the transfer of information may become temporally compartmentalised \cite{Huerta1996, Hölscher1997, Hyman2003}. This neuronal coherence between brain regions may, therefore, reflect a period of universal inter-regional communication, perhaps serving an important role in the interconnectivity of multiple brain regions at the same time (for a summary see \cite{Axmacher2006}).

Ranges of network oscillations have been found to occur within the HF and are proposed to serve an important process in the formation and retrieval of memories. It is conceivable that if neuronal activity occurs at a temporal resolution that is modifiable by spike-timing dependant plasticity i.e. LTP, then increasing the amount of synchrony between these functionally connected synapses would facilitate neuronal plasticity \cite{Dan2006, Huerta1996, Hölscher1997, Hyman2003}. The majority of hippocampal research has focused on the roles of theta and gamma rhythms in memory processing. The robust link between theta and memory has been debated for many years through numerous studies \cite{Berry1978, Givens1990, Berry2001, Buzsáki2012, McNaughton2006, Rawlins1979}. Experimental manipulation has shown that disruption to
1.4. Neuronal oscillations and their role in memory

theta rhythms produces robust deficits in spatial and working memory (McNaughton et al. 2006) and that application of bursting-like stimulations at particular phases of the theta cycle can result in potentiation/depression in synaptic connectivity (Huerta and Lisman 1996; Hölscher et al. 1997; Hyman et al. 2003).

The HF receives multi-modal inputs from the EC and forms associations and stores these processed events as memories (Eichenbaum 2000). Since the function of the HC is to not only store but also retrieve memories, it is important that the system can separate these two functions in order to minimise interference. Multiple theories have been proposed as to how theta is involved in these processes. Buzsaki proposed the first link, where he described the formation of memories as a two-stage process. He suggested that during periods of theta, memories were acquired and once these rhythms had subsided, these memory traces would then undergo a consolidation process that was independent of theta, relying on sharp-wave activity (Buzsáki 1989).

The Hasselmo model later proposed that the encoding and retrieval or memories was also a two-stage process, but both phases occurred during periods of theta (Hasselmo et al. 2002). Further, this model suggested that encoding and retrieval occur on different phases of the theta cycle, with encoding taking place at the theta trough and retrieval at the peak, relative the theta activity recorded at the hippocampal fissure (see Figure 1.5). During the trough of theta, CA1 receives a relatively weak input from CA3 and strong input from EC, resulting in the facilitation of synaptic responses. In contrast, during the theta peak the EC input is relatively weak; however, the synaptic strength of the Schaffer collaterals is increased, and therefore is likely to represent a retrieval state (Hasselmo et al. 2002; Hasselmo 2005; Brankack et al. 1993). The Lisman model develops from these earlier hypotheses and supports theta involvement in the encoding of memories but also adds the faster gamma component. This model suggests that memories (or elements of a single memory) are repeated as embedded sequences of gamma activity, which can ‘ride’ the theta wave as ‘packets’ of 7±2 gamma cycles (Lisman and Idiart 1995). With respect to these models, it has also been suggested that disruption of theta leads to memory impairment through interference of old and new memories, resulting in conflict between the two-stage process.

In addition, theta rhythms are known to be important for the control of spatially sensitive CA1 pyramidal cells, and are involved in the generation of a cognitive map. Activity within subsets of hippocampal pyramidal cells can be spatially correlated within a spatial scene to provide an internal map of that environment. Hippocampal place cells are CA1 pyramidal cells that fire specifically when an animal enters a particular location within their environment. The firing rates of these PCs are highly correlated with the underlying theta cycle, with lesions to the medial septum resulting in significantly reduced capacity for PCs to encode space (O’Keefe and
1.4. Neuronal oscillations and their role in memory

Figure 1.5. Hasselmo model of hippocampal encoding and retrieval of memories during theta rhythms. The Hasselmo model proposes that encoding and retrieval of memories occur during two distinct phases of the theta cycle. Hasselmo proposes that during the theta trough (as recorded at the hippocampal fissure), there are strong EC inputs to CA1 and relatively weak CA3 inputs, and therefore it favours the encoding of new memories. During the theta peak (as recorded at the hippocampal fissure), the EC inputs are now weak compared to the strong inputs from CA3 via the schaffer collaterals, and that this favours a retrieval stage. Images adapted from Hasselmo et al. (2002).
1.4. Neuronal oscillations and their role in memory

Dostrovsky, 1971; Ekstrom et al., 2003; Leutgeb and Mizumori, 1999). Place cells do not only encode place, but also able to encode the animal’s position in relation the rest of the environment, past and future positions through phase-locking to the theta rhythm (O’Keefe and Recce, 1993; Skaggs et al., 1996).

When an animal first enters a place field (the area within the environment in which a place cell is active), it initially fires during the later phase of the theta cycle. However, as the animal advances through the place field and its environment, the PC fires at progressively earlier phases of the theta cycle. This process is known as theta-phase precession and allows for the animal to represent its current position in relation to past and possible future positions in relation to the environment (see Figure 1.6) (O’Keefe and Recce, 1993; Skaggs et al., 1996; Huxter et al., 2003).

The relative phase at which a neuron fires in relation to the underlying theta rhythm is known to influence LTP/LTD induction. Stimuli that arrive at the theta peak (as recorded near to the cell layer) facilitate the induction of LTP, whereas stimuli arriving at the trough induce LTD (Huerta and Lisman, 1996; Hölscher et al., 1997). As such, it is likely that the timings of spikes in relation to the on-going theta rhythm are more informative than either spike times or LFP phase alone (Montemurro et al., 2008; Kayser et al., 2009). In addition, if spike-timing in relation to the on-going rhythm is an important mechanism for synaptic communication, then there must be system that allows for new stimuli to be locked to the same rhythm. This is known as ‘theta-phase reset’, which ‘resets’ the signal and allows for new incoming stimuli to become phase-locked to the theta cycle (Givens, 1996).

As discussed earlier, gamma rhythms typically occur during periods of theta. Whilst theta rhythms are thought to be involved in spatial navigation, gamma rhythms are involved in mnemonic recall, encoding and combining of memory components, and attention (Montgomery and Buzsáki, 2007; Lisman and Buzsáki, 2008; Gregoriou et al., 2009; Benchenane et al., 2011). They are also important for modifying neural connections, where the gamma-theta frequencies allow for small ‘windows of opportunity’ to induce synaptic plasticity (Axmacher et al., 2006; Dan and Poo, 2006). Whilst faster frequency bands, such as theta and gamma, tend to be associated with smaller local networks, slower oscillations are associated with neuronal coherence at the level of brain regions (Csicsvari et al., 2003; Steriade et al., 1991). During periods of immobility and deep sleep, network oscillations in the delta/slow-wave frequency bands become much more prominent. Current research suggests that these two distinct bands serve two different but complementary roles in the formation and retrieval of memories (Buzsaki, 1998).

During slow-wave sleep (SWS) there is a global increase in coherence between distant regions of the brain. This represents an increase in neuronal synchrony and
1.4. Neuronal oscillations and their role in memory

Figure 1.6. Theta phase precessions. Theta phase precession is a term used to describe the relative changes in firing rates of place cells as the animal navigates through its environment. When the animal is in the centre of the neurons place field then the firing rate is maximal at the theta trough. As the animal navigates from this point it will enter and leave subsequent place fields, mapping the entire environment. As it leaves the centre of one place field and begins to enter the next, the firing rate of the initial field will decrease and move to an earlier phase of the theta cycle, with the firing rate of the next field increasing and advancing in theta phase. For example, when the animal is in the centre of the green place field the firing rate is maximal and occurs on the trough of theta. The firing rate of the next place cell (blue) occurs during a later phase and is reduced compared to the first. Through this combination of place field phases and firing rates, theories suggest that an animal can form an internal cognitive map and therefore have a sense of environmental/spatial awareness.
likely results in the modification of synaptic connections (Moruzzi and Magoun, 1949). In addition, delta oscillations are prominent during periods of development and are found to be disrupted in developmental disorders, such as in schizophrenia (Keshavan et al., 1998). And therefore, it is possible that the long-range coherence and connectivity between regions of the brain may serve to facilitate the formation and consolidation of our memories (Born et al., 2006; Stickgold and Walker, 2005; Diekelmann and Born, 2010; Molle and Born, 2011; Walker and Stickgold, 2004).

During SWS a number of slow and faster oscillations occur, namely, sharp-waves, ripples and spindles (Yun et al., 2002; Axmacher et al., 2006; Csicsvari et al., 1999; Skaggs et al., 1996). These faster frequencies are generated during periods of delta activity and are thought to be vital for the consolidation of memory (Buzsáki, 1989). During periods of deep sleep and immobility, a subset of CA3 cells become disinhibited, leading to synchronous population discharges, resulting in the generation of sharp-waves (Buzsáki et al., 1983; Axmacher et al., 2006). These discharges, via the Schaffer collateral projections, activate CA1 pyramidal cells. This results in the generation of similar bursting activity, which in turn activates inhibitory basket cells. This leads to the generation of a recurrent inhibition network between the CA1 pyramidal cells and inhibitory basket cells (Buzsáki et al., 1983; Axmacher et al., 2006). This high frequency, bursting activity results in the concurrent activation of approximately 40,000-60,000 neurons per event and generates a very strong neuronal output. Various studies have concluded that this activity is optimal for the induction of long-lasting increases in synaptic efficiency and as a result is likely to serve as the mechanism for memory consolidation to the neocortex (Buzsáki, 1989; Yun et al., 2002).

In response to the clear separation of function across neuronal oscillations, a two-stage encoding/consolidation model of memory has been proposed (Buzsáki, 1998; Skaggs et al., 1996). This two-stage model proposes that theta oscillations represent an ‘online’ acquisition state and during periods of rest and deep-sleep, the HC switches to an ‘offline’ state with a subsequent increase in delta and ripple activity, resulting in long-term increases in synaptic efficiency, and therefore consolidation of these memories to the neocortex (Buzsáki, 1998; Frankland and Bontempi, 2005; Axmacher et al., 2006; Skaggs et al., 1996). In support of this theory, researchers have identified that the neuronal firing activity produced during periods of exploration and learning is ‘replayed’ during periods of delta (Buzsáki, 1998; Wilson and McNaughton, 1994; Lee and Wilson, 2002). In addition, reactivation of neurons during sleep is much greater in terms of number compared to the number of neurons active during exploration, and therefore is more conducive to memory consolidation. As such, it is likely that sharp-wave ripples may serve to convey information to the neocortex for long-term consolidation of memories (Gais et al., 2002; Buzsáki, 1998).
1.5 Alzheimer’s disease associated changes in network oscillations

Interestingly, until recently, it has been assumed that rhythmic activity is processed in a unidirectional manner through the hippocampal subfields. However, recent work has challenged this idea, providing evidence that the hippocampal system is much more complex than a serial processing loop that processes synaptic activity in a feedforward manner. For example, Jackson et al. (2014) showed that that the subiculum, which is typically thought as an output structure, provides strong inputs to ‘earlier’ regions of the hippocampal system during theta rhythms. In fact, they demonstrate that the subiculum is capable of entraining spiking activity within CA3 during these network oscillations (Jackson et al., 2014). This supports earlier anatomical data that both pyramidal and inhibitory in CA1 receive strong excitatory and inhibitory connections from the subiculum (Sun et al., 2014).

1.5.1 The use of EEG in Alzheimer’s disease

The EEG was first introduced in the 1930s to allow the indirect/non-invasive measurement of the brain’s electrical activity. Since we know that there are synaptic and network alterations associated with AD and its progression, it is likely that this will be detectable as global changes in synaptic activity. However, the EEG is not currently accepted as a reliable tool for detection and diagnosis of AD (Jelic and Kowalski, 2009; Fonseca et al., 2011).

Although the use of EEG for the detection and diagnosis of AD currently requires further evaluation, many studies have confirmed that there are network changes, detectable with EEG measurement, in many cases of AD. In addition, these studies also confirm that these changes are highly correlated with the onset and progression of cognitive decline in humans (Obrist et al., 1962; Johannesson et al., 1979; Kaszniak et al., 1979; Soininen et al., 1982; Rae-Grant et al., 1987; Brenner et al., 1988; Erkinjuntti et al., 1988). As such, it is imperative that further resources and research are devoted to the use of EEG as a diagnostic tool for AD. Currently, the estimated accuracy of EEG measurements for the diagnosis of the disease lies at around 80%, so with further research it may be possible to implement EEG clinically with high diagnostic confidence (Jeong, 2004; Brenner et al., 1988).

In AD there is a typical ‘slowing’ of the network rhythm that worsens with disease progression (Montplaisir et al., 1996; Hassainia et al., 1997; Jeong, 2004). Initially, during MCI, there is a general increase in the power of theta rhythms
and a decrease in the beta band. As the disease progresses, the increase in theta power is followed by an increase in delta rhythm power with a parallel decrease and subsequent loss of alpha rhythms (Jeong, 2004; Coben et al., 1985; Hier et al., 1991; Bennys et al., 2001; Brenner et al., 1988).

Interestingly, these changes are found during periods of wake and sleep, with network alterations also evident in AD models (Hassainia et al., 1997; Montplaisir et al., 1996; Jyoti et al., 2010; Platt and Riedel, 2011). Preliminary results from studies using AD models suggest that there is slowing of the theta rhythm prior to deposition of Aβ plaques (Goutagny et al., 2013). Whilst the authors suggest that this may be comparable to the MCI stage in humans, further research is required in order to validate their claims. In addition to the general slowing of network oscillations in AD, studies have also found evidence of alterations in theta rhythm during memory tasks. Studies have demonstrated that there are detectable changes in the power of theta rhythms in AD patients when they are engaged in tasks that require a high memory load. Specifically, the results indicate that there is a general decrease in theta power, suggesting that this may account for a reduction in the ability to acquire and retain memories (Wang et al., 2002; Rubio et al., 2012; Scott et al., 2012).

Whilst the research discussed above suggests that there is a clear and detectable change in network processing in AD, it is important to consider that other studies have failed to detect any AD related changes in the EEG signal (Soininen et al., 1982). However, in support of the use of EEG as a diagnostic tool in dementia, other studies have confirmed that there are other detectable changes in the rhythms associated with other diseases. For example, patients with confirmed fronto-temporal cortical atrophy show relatively normal EEG patterns or a moderate and diffuse increase in theta. Additionally, when compared to controls, FTD patients show a lack of increase in slow EEG rhythms (Jeong, 2004). As such, it may be possible that the network changes associated with different types of dementia are slightly different and, therefore, differential EEG spectral changes may distinguish types of dementia in patients (Johannesson et al., 1979; Jeong, 2004).

The clear change in theta rhythms may be at least partly due to changes in cholinergic modulation. Studies have shown that alterations to the cholinergic system can result in the slowing of EEG frequencies in healthy and diseased subjects (Coyle et al., 1983; Jeong, 2004; Platt and Riedel, 2011). Changes in cholinergic tone in AD are thought to be a key contributor to the changes found in the EEG rhythms. Acute administration of cholinergic drugs such as Donepezil, an anticholinesterase inhibitor, produce significant improvements in memory and attention and typically result in an EEG signal comparable to control patients. In support of this finding,
administration of anticholinergic drugs, such as scopolamine, induce the opposite effect, resulting in slowing of the EEG \cite{Lahiri2002, Jeong2004}. Whilst studies involving acute administration of cholinergic drugs seem to ‘reverse’ the EEG changes and restore some cognitive function, chronic administration of these drugs does not have a long-term effect. Administration of Donepezil results in a reduction of spontaneous theta power and limits the EEG deterioration, however, this effect is only evident during the early-mid stages of the disease, with no clinical benefit during advanced stages \cite{Kogan2001, Rodriguez2002}. As such, it is likely that changes in the cholinergic system contribute to AD but are not solely accountable.

1.5.2 Coherence and information analysis

Assessment of neuronal function through the use of EEG power measurement may provide useful information for diagnosis, however, this does investigate how connectivity between regions of the brain may change in AD. Some studies have conducted coherence analysis between EEG signals from different regions of the brain. Coherence between two or more regions is thought to measure how well these regions are functionally connected to one another \cite{Leuchter1987, Besthorn1994, Locatelli1998}. Although this method would provide a greater understanding of brain connectivity and builds on the results from other studies, there has been limited work assessing neuronal coherence in disease. Of the latter, one study assessed theta-gamma cross-frequency coupling in a mouse AD model and found differences as early as 1 month old \cite{Goutagny2013}. This would suggest, therefore, that there are functional changes and disconnection within the brain prior to overt AD-related pathology. However, it is important to consider that this study was conducted on \textit{in vitro} preparations and, therefore, represents a heavily reduced preparation in terms of functional connectivity. Coherence analysis between two or more regions provides some insight into inter-regional connectivity, however, coherence analysis only provides a linear measurement. As such, coherence alone is unable to determine causality between signals and, therefore, cannot determine the influence of one signal over the other. To address the latter some measure of causality is required. The ‘causality principle’ was first introduced in 1969 and allows for determination of the influence of one signal over another whilst discounting any effect arising from common inputs to both regions, e.g., rhythm generators \cite{Granger1969}. In order to calculate causality between two non-linear signals, firstly we must compute Shannon entropy values based on the signal. This Shannon entropy is typically calculated as the average ‘unpredictability’ of a given variable/response \cite{Shannon1948}. Using these values, we can determine the amount of shared information between two signals.
through the calculation of mutual information ($MI$). This builds on Shannon entropy and determines how much one signal informs us about the other, i.e. how much signal $X$ informs us about signal $Y$. However, $MI$ calculates are symmetric, that is $MI(X,Y) = MI(Y,X)$, and there is unable to infer causality. Through application of the causality principle to $MI$ measurements we can measure transfer entropy ($TE$). Transfer entropy quantifies the causal relationship between two signals. It assesses whether or not knowing the previous history of one signal ($Y$) allows for the greater prediction of the other signal ($X$) above that by knowing the history of $X$ alone. Since the application of $TE$ allows the analysis of non-linear signals, such as LFPs, the application of this method can determine and quantify the amount and direction of information flow between two functionally connected brain regions (Granger, 1969; Shannon, 1948; Schreiber, 2000). As such, the promise of this analytical technique is to determine whether and how inter-regional communication breaks down in AD and how this may evolve with disease progression.

As this study aims to develop our understanding of network changes in AD and age, it is important for us to consider the potential anaesthetic implications. In order to validate the use of anaesthesia in the study, the next section will review the use and mechanisms of action of urethane for acute preparations.

### 1.6 Urethane as the anaesthetic of choice for acute recordings in rodents

Most studies involving animal models of disease must take into account the effect of anaesthesia on brain activity. All anaesthetic agents are known to have an effect on neuronal activity and, as a result, typically have an effect on the oscillatory states of the brain. The majority of anaesthetic agents induce a dominant slow-wave/delta rhythm and, therefore, the effect of the agent must be strongly considered when planning and conducting experiments. This is particularly relevant if you want to assess AD-related changes in network oscillations.

The ideal anaesthetic agent should induce a reliable condition of behavioural unconsciousness that closely mimics the full spectrum of physiological sleep. As such, urethane is one of the only anaesthetics that matches this requirement and therefore represents the ideal candidate for assessing neuronal function and communication in health and disease (Clement et al., 2008). Under urethane anaesthesia brain activity is found to oscillate between an ‘online’ (REM) and ‘offline’ (non-REM) state, similar to those observed under normal sleeping conditions.

Studies involving urethane anaesthetised rats where LFP activity was recorded in
the EC and neocortex indicated that LFPs oscillated between an online (3-5Hz) and offline ‘deactivated’ (1Hz) state (Clement et al. 2008). However, some criticism has been received, with other studies suggesting that this cycling activity can be attributed to fluctuations in the concentrations of urethane within the bloodstream. These studies suggest that urethane metabolites decline in a progressive manner throughout the recording and therefore could result in changes in the level of anaesthesia (Nomeir et al. 1989; Sotomayor and Collins, 1990). In contrast, Clement and colleagues show that additional ‘top-up’ doses of urethane throughout the recording period had no influence on the underlying network oscillations. In conclusion, the authors suggest that the network responses are likely to be in response to actions of the drug rather than the blood concentration (Clement et al. 2008).

In further support of the use of urethane as a model anaesthetic, Clement et al. provided further evidence that urethane-induced anaesthesia not only results in cyclic network oscillations, but that the duration of each period is similar to physiological sleep (Clement et al. 2008; Borbely 1976). In addition, the peak power and frequencies, with the exception of theta, of the underlying oscillation are similar to that found in normal sleep. Differences in theta frequency, 4Hz under urethane compared to 6Hz during sleep, is likely due to alterations in theta generation under anaesthesia (Clement et al. 2008). Under normal conditions, there are two pharmacologically distinct theta rhythms. One of these is atropine sensitive, and provides input to pyramidal cell bodies, and the other is atropine insensitive and provides input at the hippocampal fissure (Kramis et al. 1975). As such, theta rhythms are generated from two distinct origins. Surgical ablation of the EC or disconnection of the EC-hippocampal input, results in complete removal of atropine insensitive theta, whilst imposing no effect on atropine sensitive theta. Under urethane-induced anaesthesia, hippocampal LFP recordings are similar to those observed following EC ablation or disconnection (Buzsáki 2002). As such, it is likely that urethane affects the atropine insensitive sub-type of the theta rhythm, which accounts for the differences in the observed theta frequency when compared to sleep (Clement et al. 2008). The blockade of atropine insensitive theta under urethane anaesthesia is not currently fully understood. However, it is possible that urethane exerts, at least to some extent, some of its effects via glutamatergic receptors in layers II and III of the EC (Amaral and Witter 1989). In addition, urethane also possibly results in the attenuation of glutamate release or has a pharmacological effect on NMDA receptor currents within the distal dendrites of the HC (Moroni et al. 1981; Buzsáki 2002).

Whilst it is evident that urethane anaesthesia results in some pharmacological changes that are not found during sleep, it is still currently the best candidate for investigating cyclic network oscillation in acute preparations. In support of
the use of urethane, Clement and colleagues found further similarities between the mechanisms involved in sleep and anaesthesia induction. Under normal physiological conditions the induction of sleep requires the release of endogenous acetylcholine from the forebrain, and activation of the ascending monoaminergic systems during wakefulness (Vanderwolf et al., 1997; Jones, 2003). Clement found that changes in the monoaminergic system did not have any effect on the LFP activity under anaesthesia, however, changes to the cholinergic ascending arousal system did result in an effect (Clement et al., 2008). As such, urethane-induced network changes are likely, at least to some extent, to be modulated by the cholinergic arousal system.

However, while there are distinct similarities between the network oscillations observed in the awake state and under urethane anaesthesia, it is important to consider the differences in the modulation of neuronal activity. Recently, a number of studies have documented the distinct functional roles of subclasses of GABAergic interneurons in the generation and modulation of network oscillations (Lapray et al., 2012; Varga et al., 2012; Katona et al., 2014; Varga et al., 2014). For example, it is now known that the three subclasses of PV-expressing hippocampal interneurons have functionally distinct roles in rhythm generation (Varga et al., 2014), and that OLM interneurons have temporal differences between the awake and sleep state (Katona et al., 2014). However, how these are affected under anaesthesia is currently unknown.

A recent study by Lapray et al. (2012) demonstrated that different subtypes of interneurons have distinct roles in: a) the control of network excitability, and 2) the coordination of cell assemblies during behavioural states. While these differences have been observed in the awake behaving animal, it is currently unknown whether anaesthetic agents affect these mechanisms. Interestingly, Varga et al. (2012) demonstrated differences in the temporal inputs of PV and OLM interneurons to hippocampal neurons in the awake state and under urethane anaesthesia during periods of theta activity. They revealed that there was a phase shift in the timings in which PV interneurons fired in relation to theta between the awake and anaesthetised state, and a loss of gamma modulation in OLM interneurons when under urethane anaesthesia. In addition, they demonstrated that the firing rate of OLM interneurons in relation to SWR activity altered between states, with the firing rate increasing when in the awake state, but decreases under urethane anaesthesia.

Therefore, it is evident that the control of network oscillations is complex and the mechanisms and dynamics are likely to be affected by anaesthesia. However, whilst we appreciate that urethane-induced anaesthesia does not perfectly mimic physiological sleep, there is significant scientific support that it results in similar effects and therefore is still the most appropriate anaesthetic agent for use in acute
1.7 Specific research aims

To date there has been little research that has helped to determine how hippocampal function alters as a function of age and disease. It is now more important than ever to focus our research strategies to investigate how and when neuronal dysfunction occurs, and how this may change with disease progression. There has been some, albeit limited, research that has provided an insight into how hippocampal activity changes in disease, yet we are still unable to correlate the onset and progression of synaptic deficits with the underlying pathological state of the brain. Of the studies reviewed above that assessed neuronal dysfunction in AD, there have been a variety of reported results, most likely due to differences in the preparation (in-vitro or in-vivo), animal model (e.g. APP, PS1/APP, Tau, 3xTgAD) and the variety of HF regions that have been assessed.

In order for the results from scientific research to be translated into a clinical setting, it is important for us to investigate these synaptic changes further, particularly at the early stages of disease. Importantly, we need to probe the physiology of the hippocampal system in vivo and investigate the clinical significance of changes in LFPs in disease.

Therefore, in this study, I aimed to conduct an in-depth investigation to assess neuronal function as a function of age and disease. I will, for the first time, investigate the synaptic integrity of the final processing pathway of the HF, field CA1 and subiculum, which is a prime but substantially under-investigated target of AD. In order to determine the synaptic changes associated with the disease, it is vital to ensure that the model of choice mimics the human form of AD as closely as possible. Therefore, I will use the 3xTgAD mouse model of AD, which displays the full pathological and behavioural phenotype similar to that found in AD patients.

Specifically, the aims of the my study are:

1. To determine the onset and development of synaptic deficits within the hippocampal circuitry in 3xTgAD mice in-vivo through the assessment of short- and long-term plasticity between CA1 and subiculum.

2. To determine how the spectral changes in network oscillations may change as a function of age and disease, and how this may correlate with synaptic and information deficits.
To assess the information carrying capacity of network oscillations between these regions through the use of information theoretic analysis methods.

1.8 Alternative format structure

This thesis is written in the alternative format structure to allow for the timely dissemination of my work through publication. Each results chapter is written as a stand-alone research paper. It is my intention, upon completion of my PhD, to submit Paper 1 to PLOS ONE, and Paper 2 to Journal of Neuroscience.

Below is a brief description of the work contained within each chapter and a declaration of author’s contributions.

1.8.1 Paper 1

Title: Characterisation of neuronal deficits in the CA1-subicular pathway of the 3xTgAD mouse in vivo.

Authors Daniel Squirrell, Sarah Fox, Marcelo Montemurro, John Gigg.

Declaration of author contribution: The body of work and analyses were conducted by Daniel Squirrell under the supervision of John Gigg and Marcelo Montemurro. Analyses were undertaken by Daniel Squirrell using programs developed and written by Sarah Fox, and modified by Daniel Squirrell.

Paper overview: Here, I characterise the neuronal changes in the CA1 and subiculum pathway, and determine how these are associated with age- and AD-like pathology progression in the 3xTgAD mouse. This paper provides the first study to assess the impact of AD-like pathology on the basic synaptic connectivity, and correlates this with alterations in cognitive performance.

1.8.2 Paper 2

Title: An in vivo assessment of the information carrying capacity of the CA1-subicular pathway in the 3xTgAD mouse.

Authors: Daniel Squirrell, Maria Constantinou, Marcelo Montenurro, John Gigg.

Declaration of author contribution: The body of work and analyses were conducted by Daniel Squirrell under the supervision of John Gigg and Marcelo
Montemurro. Analyses were undertaken by Daniel Squirrell using programs developed and written by Marcelo Montemurro and modified by Daniel Squirrell and Maria Constantinou.

**Paper overview:** Through the implementation of novel information theoretic techniques, I determine how the information carrying capacity of the CA1-subiculum network alters as a function of age and AD-like pathology in the 3xTgAD mouse. For the first time, I quantify the breakdown in neuronal communication during different network oscillations, and correlate this with changes in neuronal connectivity and cognition.
Chapter 2

Methods

2.1 Animals

Experiments were conducted on male 3xTgAD and C57/129sv mice, where the original breeding pair was kindly donated by the LaFerla lab (University of California, USA) (Oddo et al., 2003). The 3xTgAD mouse is a murine model of AD carrying the human transgenes APP\textsubscript{SWE}, PS1\textsubscript{M146V} and Tau\textsubscript{P301L}. Briefly, to generate the model the human transgenes for APP\textsubscript{SWE} and Tau\textsubscript{P301L} were microinjected into single cell embryos of homozygous PS1\textsubscript{M146V} mice. The offspring were then backcrossed multiple times in order to create stable homozygous offspring carrying all three genes, resulting in the generation of 3xTgAD mice. The 3xTgAD colony was then maintained ‘in-house’ through pairing of homozygous individuals, followed by confirmation of genetic stability by genotyping subsets of mice to confirm the presence of the three genes. Control mice (C57/129sv) were of the background strain of the PS1 knock-in mouse, and therefore also that of the 3xTgAD mouse. Similarly to the 3xTgAD mice, control mice were housed in groups of 4-6 and maintained ‘in house’.

Mice were housed with same sex and genotype cage-mates of 4-6 individuals in a pathogen free environment on a 12hr light:dark cycle (lights on at 7am) with food and water available \textit{ab-libitum}. All experimental procedures were performed in accordance with the Animals (Scientific) procedures Act 1986 (UK).

Data in this study were collected from three cohorts of mice aged: 3-4 months, 6-7 months and 9-10 months. This allowed a full profile of the development and progression of synaptic dysfunction from a young age, prior to overt AD-like pathology and behavioural decline, through to an age where cognitive decline and pathological markers are typically present (Mastrangelo and Bowers, 2008 | Billings et al., 2005 | Oddo et al., 2003 | Davis et al., 2013b, a | 2014). The study was initially designed so
that a late-stage could also be assessed by obtaining data from an aged cohort of 16 months; however, we subsequently decided to remove this group due to their poor survival under anaesthesia.

2.2 Anaesthesia and surgery

Anaesthesia was induced via intraperitoneal (i.p) injection of urethane (1.5-1.7g/kg of 30% w/v solution prepared in 0.9% Saline, Sigma, UK). We found that the required dose of anaesthesia was strain- and age-dependant and therefore were altered accordingly. If complete areflexia was not induced after 40 minutes had passed since the original injection, additional anaesthesia was administered by means of a ‘top-up’ dose (50µl of 10% w/v urethane solution prepared in 0.9% Saline). This would continue every 30 minutes until the correct depth of anaesthesia had be induced. Breathing and survival under urethane was aided by performing a tracheotomy (Moldestad et al., 2009). Core body temperature was maintained at 37°C for the duration of the experiment through use of a rectal thermometer placed underneath the abdomen and a homoeothermic blanket.

Mice were mounted and fixed in a stereotaxic frame (Kopf 1430, USA), comprising of ear and mouse adaptor in order to immobilise the head prior to surgery. The head was orientated in a position relative to the mouse brain atlas (Franklin and Paxinos, 2007) to aid with electrode targeting.

A midline incision was made and the scalp retracted to expose the skull. Debris and tissue was then scraped away from the skull until Bregma and Lambda were clearly identifiable. The distance between Bregma and Lambda was measured for each mouse prior to marking of craniotomy positions; any difference to that reported in the mouse brain atlas (4.2mm) was taken into consideration by adjusting co-ordinates for craniotomies proportionately in order to improve accuracy of placements. Thereafter, co-ordinates for craniotomy and electrode placement were marked onto the skull, relative to Bregma and the midline for CA1 (Bregma: -1.5mm, ML: 1.7mm) and subiculum (Bregma: -2.8mm, ML: 1.7mm)

Craniotomies were drilled using a 0.9mm drill bit (Fine Science Tools, Germany) and a high-speed hand held drill (Foredom, USA). Care was taken to ensure that overlying blood vessels and cortex remained intact during this process. Saline-soaked cotton wool was then placed in the first craniotomy to keep it moist until the second was complete. A small slit was then made in the overlying dura with a small needle to aid the smooth insertion of the recording and stimulating electrodes.
2.3 Electrode placement

Initially, two multi-electrode recording arrays (MEAs) were placed into CA1 and the subiculum for spontaneous recordings. The particular arrangement of recording contacts was chosen in order to span the cell layers of each region. Both electrodes consisted of thirty-two 413μm² contacts spanning over two shanks (16 contacts per shank) with 100μm vertical spacing between contacts and 500μm horizontal spacing between shanks (A2x16-10-100-500-413, NeuroNexusTech, USA) (see Figure 2.1). The CA1 recording electrode was inserted at a compound angle of 20° and lowered by 2.25mm from the surface of the cortex. The subiculum electrode was then inserted at a compound angle of 25° (from vertical) and lowered by 2.38mm. The compound angle was chosen to allow for easy access of both electrodes and to accommodate for the curvature of the subiculum (so that the vertical axis of the electrode was matched with the main dendritic orientation of subicular pyramidal cells; see Figure 2.1). Note that both recording electrodes were coated in Vibrant® CM-DiI (Life Technologies, UK) cell-labelling solution to allow for visualisation of electrode placement through use of post-hoc fluorescence microscopy.

To help to determine the exact position of recording electrodes lesions were created by passing a positive current through two contacts on the CA1 electrode (30μA for 6 seconds) upon completion of spontaneous recordings (Townsend et al., 2002). Note that the production of these lesions has a minimal effect on the integrity of the network (Townsend et al., 2002). This electrode was then removed and replaced by a bipolar stimulating electrode (twisted 125μm diameter Teflon-insulated stainless-steel wires; Advent RM, UK). The stimulating electrode was positioned at the surface of the cortex as to avoid the location of the lesions produced earlier and slowly lowered until the CA1 pyramidal cell layer was reached (Figure 2.2).

2.4 Spontaneous recordings and data acquisition

Spontaneous spiking and LFP activity was recorded for a period of at least thirty minutes from each contact on both electrodes. Data was recorded on a Recorder64 system (Plexon Inc, USA) with reference to a common ground. Signals were amplified through a headstage of fixed x20 gain and then to a variable-gain amplifier, typically set to provide a total gain of x2000.

LFP signals were sampled at 2KHz using a 12-bit A/D convertor and low-pass filtered (Butterworth, 1 pole) at 250Hz. Spiking activity was sampled at 40KHz per channel with a high-pass filter of 300Hz (Butterworth, 1 pole). Spikes were
2.5. Stimulation protocol

2.5.1 Insertion of stimulating electrode

After completion of the spontaneous recording session the CA1 recording electrode was removed and replaced by the bi-polar stimulating electrode, initially positioned on the surface of cortex overlying CA1. As the stimulating electrode was lowered to the CA1 target paired-pulse stimulation (PPS) was applied (paired-pulse interval recorded as discrete wave shapes (500µs pre-threshold, 800µs duration) that exceeded a manually determined voltage threshold chosen to improve the signal-noise ratio. All data were recorded and saved for offline analysis.

Electrical stimuli were delivered by a constant-current stimulator (DS3, Digitimer, UK), which was triggered by analogue 5V square wave pulses from a National Instruments PCI card (PCI-6071E). Timings and types of stimuli to be delivered were controlled through custom designed programs written in LabVIEW (v8, National Instruments). Stimulus duration was fixed at 200µs throughout each protocol.

During the recording session, activity from each contact could be visualised (four at any one time) in more detail by monitoring activity through a series of oscilloscopes (HAMEG Instruments GmbH, US). Spontaneous and evoked field and spiking activity were typically monitored in order to help determine whether the electrodes were correctly positioned in their target regions.

2.5 Stimulation protocol

Figure 2.1. Placement of recording electrodes. (a) Visual representation of the configuration of a 2x16 recording electrode. (b) Two sets of 2x16 recording electrodes were lowered into target areas CA1 and Subiculum to obtain spontaneous recordings of extracellular LFP and spiking activity. (Hippocampal image adapted from Franklin and Paxinos (2007)).
50ms, 3s between pairs) until the CA1 pyramidal cell layer was reached and a typical subicular LFP response profile established (see Figure 2.2).

A typical extracellular subicular evoked LFP consisted of an initial negative event (presumed EPSP; latency to trough ~5ms), followed by a positive event (presumed IPSP). Reversal of the initial LFP becomes evident on contacts positioned further towards the tip of the shank, together with a slightly delayed negative event, presumably caused by back-propagation into the dendrites (e.g., for CA1 see Leung and Peloquin (2006)). Whilst lowering the CA1 stimulation electrode we frequently observed a very fast (1-2ms) negative event representing stimulation of the alveus, leading to antidromic activation. As the tip of the stimulator was lowered further, the antidromic response faded and a later (5-6ms) orthodromic response became evident.

Figure 2.2. Insertion of stimulating electrode and typical subicular LFP response profile. Stimulation electrodes were slowly lowered until the dorsal CA1 pyramidal cell layer was reached. Application of a stimulus to CA1 resulted in monosynaptic activation of the subiculum, generating a laminar response profile (right).
2.5.2 Assessment of functional neuronal connectivity

Once a stable characteristic laminar response profile was achieved along the full axis of the subicular electrode an input-output (I/O) curve was plotted by application of low-frequency stimulation (LFS) over a range of current intensities (0.33Hz, 20 repeats at 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450 and 500 µA). Latency, slope and amplitude values were measured and recorded. Average response amplitudes were calculated for each stimulation intensity and stimulating current was then set to elicit half the maximum response for all subsequent stimulation (typically 150-200 µA).

2.5.3 Assessing short-term plasticity using paired-pulse stimulation

A paired-pulse protocol was utilised in order to probe the network’s ability to express short-term synaptic potentiation. Paired-pulses were delivered at various intervals (20, 50, 100, 200, 500 and 1000ms) with 20 repeats for each. Facilitation could be identified by an increase in the response to the second pulse (P2) compared to the first (P1).

2.5.4 Low-frequency train stimulation

In order to assess the additive effects of repeated stimulation in terms of plasticity and re-entrance of activity around the hippocampal circuitry [Kloosterman et al., 2003, 2004], a train of low-frequency stimuli was applied (20 stimuli at 5Hz or 10Hz with 5 repeats). These frequencies were chosen so that a comparison could be made against those used during PPS and were predicted not to result in any long-lasting changes in the network circuitry [Kloosterman et al., 2003, 2004]. LFP responses were analysed further in detail for pulses 1, 5, 10 and 20 of each stimulus train.

2.5.5 Long-term potentiation

We further assessed the ability of the neuronal connections between CA1 and subiculum to demonstrate evidence of LTP by means of a HFS protocol. A baseline recording was taken, prior to application of the HFS, through application of 50ms PPS (30s ISI) for a period of 15 minutes. A HFS (200Hz burst of 20 pulses, with 5 repeats separated by 2s) was then applied to CA1, followed by a series of 50ms PPS for a period of 60 minutes post HFS (120 repeats, 30s ISI) [Commins et al., 1998; Craig and Commins, 2006].
2.6 Perfusion and storage of brain tissue

Upon completion of electrophysiological recordings an overdose of urethane was administered and mice were transcardially perfused with 0.9% saline nitrate until the animal was cleared of any blood (identified when the fluid leaving the right atrium ran clear). The saline buffer solution was then substituted with 4% paraformaldehyde solution (dissolved in 1x PBS) (PFA) until the animal was fully fixed. Fixed brains were then removed from the skull and stored in 4% PFA for \(\sim 3\) days prior to sectioning. For long-term storage of tissue, brains were cyroprotected by sinking them in a 30% sucrose solution (to remove any water from the tissue) prior to placing them in antifreeze solution and stored at \(-20^\circ C\).

2.7 Overview of experimental protocol

1. The animal was anaesthetised, placed in a stereotaxic frame and prepared for insertion of electrodes.
2. Both 2x16 recording electrodes were slowly lowered into their target regions; CA1 and Subiculum, so that they were perpendicular to the main axis of the pyramidal cell layers.
3. The preparation was left to settle for \(\sim 30\) minutes to account for any slight displacement of brain tissue by electrode insertion, followed by 30 minutes of spontaneous LFP and neuronal recordings.
4. Lesions were produced in CA1 and the CA1 recording electrode removed.
5. A stimulating electrode replaced the CA1 recording electrode and was slowly lowered until the CA1 pyramidal cell layer was activated, producing a clear response profile in subiculum.
6. The stimulation protocol was then conducted.
7. Upon completion of the above, the electrodes were removed, the animal transcardially perfused and the brain removed and stored in 4% PFA.

2.8 Histology

To conduct histological analysis, stored tissue was washed in 1xPBS, the cerebellum removed, cut down the midline (to separate the hemispheres) and sectioned on
2.9 Data analysis

2.9.1 Electrode localisation

In order to conduct this study, it was important to reliably identify the position of individual contact sites on the recording electrodes within hippocampal sub-regions. To increase the reliability and accuracy, we used two methods that enabled me to map the contact positions through the hippocampus. Firstly, recording electrodes were coated in a fluorescent cell labelling dye (CM-DiI) prior to insertion into the brain. This dye attaches to cell membranes and lipids, which, during insertion, leaves a visible trace of the shanks tracks. However, there were some limitations associated with the sole use of the CM-DiI as in some cases dye tracks were either absent or fragmented. To overcome this limitation we made use of the lesions created on the CA1 recording electrode and the evoked response profile from the shank positioned within the subiculum. We then combined images of electrode tracks and lesion positions with the laminar subicular response profile to reliably map the precise individual recording site positions (see Figure 2.3.).
Figure 2.3. Confirmation of electrode localisation. (A) Shows examples of two lesions (indicated by red arrows) within CA1, one within the stratum radiatum and second at the hippocampal fissure. (B) and (C) show DiI traces of correctly aligned CA1 and Subicular recording electrode tracks. (D) Example of subicular evoked response profile following CA1 stimulation, with a typical subicular EPSP marked (dotted box). Top of this shows a ‘typical’ response profile with reversal of the EPSP in the DG (bottom response).
2.9.2 Channel selection for further analysis

Field data obtained during the 50ms paired-pulse stimulation protocol were visualised in Neuroexplorer (Version 4.111, Nex Technologies, USA) and then transferred into Matlab (Version 7.10, Mathworks, USA). Stimulus times were recorded into one event channel so Pulse 1 and pulse 2 timestamps were extracted from this using a custom made Matlab script. Perievent histograms were then calculated (triggered to either the 1st or 2nd paired-pulse timestamp, 20 presentations for each interval tested) for all channels, mapped to the MEA site configuration. An example of the laminar response profile with the mean EPSP responses to the 1st and 2nd pulse during the 50ms PPS overlaid is presented in Figure 2.4, based on the summed EPSP at each contact position on the electrode. The electrode that was positioned within the cell layer and showed a characteristic downward-going EPSP was chosen for subsequent electrophysiological analysis.

Figure 2.4. Example of subicular evoked EPSP and PPF. Left: Example of a typical subicular evoked EPSP response profile. Right: Illustration of paired-pulse facilitation. Black line represents the response to the first pulse and grey line represents the response to the second pulse (50ms paired-pulse interval).
2.9.3 Analysis of the stimulation data

Input/Output curve

The amplitude, slope and latency of the evoked EPSP responses were calculated using custom designed Matlab scripts. Firstly, an average EPSP response from the 20 repeats was calculated and displayed for the desired contact on the electrode. Based on this, two manually selected data points around the peak of the response and all subsequent waveforms were normalised to these. This ensured that all waveforms were zeroed to this position of the waveform in order to calculate a more accurate amplitude value (see Figure 2.5).

After zeroing the baseline four more waveform positions were selected; two to measure the slope of the initial downward deflection (on the most linear part of the initial response) and two additional points around the response trough (the most negative value between the points was taken as the peak amplitude of the field EPSP). The Matlab program used the points marking the slope and peak response on the average EPSP in order to calculate the slope, latency and amplitude values for each of the 20 individual repeats. To ensure that the program functioned correctly the output was compared to manual analysis at regular intervals. These calculations were repeated for all stimulation intensities (50-500µA) during the I/O curve stimulation protocol in order to assess the basic connectivity between CA1 and subiculum and how an increase in stimulation intensity affected the properties of the subicular response.

Paired-pulse stimulation

In order to measure properties from the data obtained during PPS, another custom designed Matlab script was used to separate the timestamps of the first and second pulse in each of the repeats. The same analysis was conducted on the data for EPSPs to pulse 1 and then to pulse 2. Firstly, an average EPSP was generated for each intra-pulse interval period; zeroed and a cursor point marked in order to calculate the slope, latency and amplitude from each of the 20 repeats.

All paired-pulse values in response to the second pulse were normalised to those measured to the first pulse and then expressed as a percentage change. An increase in response to the second pulse (evidence of synaptic facilitation) was indicated by a positive percentage change.
Figure 2.5. Obtaining measurements from subicular EPSPs. (A) A typical subicular field EPSP. Note the initial negative deflection in the response defining an influx of ions into the cell upon dendritic stimulation. This differs to an intracellular EPSP that would show dendritic activation as a positive going deflection, as a direct measure of the influx of positive ions. Markers are placed around the peak immediately following stimulation (stimulation marked by *) (blue x), around the negative going response to measure slope values (red x), and around the trough of the response in order to measure the most negative value of the EPSP (green x). Peak values (blue x) are measured in order to 'zero' the waveform so that the peak of the response lies at zero (B). This ensures that all trough measurements (green x) are standardised and therefore comparable.
Stimulation train analysis

To assess the additive effects of repeated stimulation in terms of short-term synaptic plasticity and re-entrance of activity around the hippocampal circuitry, a train of low-frequency stimuli was applied (20 stimuli at 5 or 10Hz, each repeated 5 times).

Again, response values were obtained from each of the 20 stimuli over the 5 repeats in a similar way that described in the sections above. Properties of the EPSP waveform were measured for responses to both the 5 and 10Hz stimulation and represented as raw values and as a percentage change to first pulse.

Long-term potentiation

Similarly to the PPS analysis, slope and amplitude measurements obtained post-HFS were normalised to those recorded pre-HFS to determine whether LTP had occurred.

All data were analysed using Prism 5 (GraphPad, UK). Two-way ANOVA, or one-sample t-tests followed by post-hoc comparisons were applied to identify any pair-wise genotype difference in each of the measures. (see Results section for greater detail).

2.9.4 Current Source Density (CSD) Analysis

Extracellular recordings using MEAs within a large neuronal population can provide a high-resolution view of synaptic activity and the spatiotemporal properties of synaptic current flow can be visualised and mapped through use of CSD analysis. The method of CSD using high-density MEAs analysis produces a detailed, anatomically aligned map of synaptic activity. This is very difficult to accomplish with intracellular recordings due to the necessity to record from the dendritic regions of cells with an electrode whose tip width is the same order of size as the dendrite.

The algorithms used for this type of analysis rely on the activity of the neuronal population being recorded from electrodes that are aligned perpendicular to the main axis of the principal cell layer and that the synaptic flow and tissue composition is homogenous throughout the region of interest. As such, this makes the subiculum an ideal region of the brain to conduct CSD analysis.

Responses to P1, P5, P10 and P20 from the stimulation train of each the 5 and 10Hz trains were subjected to 1D CSD analysis. CSD analysis was performed by
2.9. Data analysis

estimating the second order spatial derivative of the laminar field potential profile (see Equation 2.1 below) (Nicholson and Freeman 1975; Kloosterman et al. 2004).

\[
CSD(h,t) = \frac{\sigma(h)(\Phi(h - n\Delta h, t) - 2\Phi(h, t) + \Phi(h + n\Delta h, t))}{(n\Delta h)^2}
\] (2.1)

CSD (h,t) is the CSD at a fixed time (t) and depth (h), \(\sigma(h)\) is the tissue conductivity (assumed to be constant), \(\Phi(h,t)\) is the field potential at a given time and depth, \(\Delta h\) is the distance between the contacts on the electrodes (100\(\mu\)m for present recordings) and \(n\) is the spatial smoothing value. The smoothing value (n) was set to ‘4’ to generate a smooth CSD profile.

Producing a peri-event histogram in Neuroexplorer created single pulse laminar response profiles that were subsequently analysed using a custom designed 1D CSD Matlab script (based on Equation 1) for pulse 1, pulse 5, pulse 10 and pulse 20 choosing contacts that were positioned within subiculum.

CSD values were generated as arbitrary units and were scaled to maximum/minimum values within each animal through P1-P20 measurements. Current sources were illustrated in red, sinks in blue and neutral regions green (see Figure 2.6).

The Matlab program incorporated a linear interpolating function that would estimate the CSD values between each of the measured values for contacts in the dorsal-ventral axis. This produced a smooth representation of current flow through the system.

After visualising each of the CSD response profiles for each mouse across age and genotype, it became apparent that there were five common features found within the profile. Each of these was presumed to be (see Figure 2.7):

1. Current sink/source representing initial depolarisation in Subiculum cell layer across 1-2.
2. Current sink/source likely to represent feedback inhibition across 3-4.
3. Possible re-entrance at 5.

Measurements were then taken from each of these characteristic CSD events from each animal at each age and genotype in order to investigate if there was a change in these components as a function of age and AD-like pathology (see Figure 2.7).
2.9. Data analysis

Figure 2.6. Illustration of CSD profiles through the stimulation train. Average CSD profiles were constructed to P1, P5, P10 and P20 of the stimulation train. Each image was scaled to the most positive value through all 20 pulses, as such it is possible to note how the sink/source current changes as the stimulus number advances.

Figure 2.7. Common spatial components present in CSD profiles. Five components were common throughout the majority of CSD response profiles. These were presumed to be: 1) Excitatory sink, 2) concurrent excitatory source, 3) inhibitory source, 4) concurrent inhibitory sink and 5) hippocampal re-entrance. Note that this is an example profile from the first pulse in the train.
2.9.5 State separation

Urethane anaesthesia induces a state of unconsciousness similar to that found during sleep ([Clement et al., 2008](#)). As a result, we can distinguish periods of theta and delta/SWS-like oscillations in brain activity. In order to assess the impact of AD on network oscillations, I had to reliably separate periods of theta and delta from the spontaneous LFP activity.

Initially we had to determine the frequency bands in which these network oscillations lie within our recordings. As this seems to vary throughout the literature and is conditional to the type of preparation involved (e.g. awake or anaesthetised recordings), we made use of custom designed Matlab scripts to determine the bands that were appropriate to this specific investigation. To address this issue, we created average fast Fourier transforms (FFTs) for each channel for each animal. We then created an average within-region FFT and visualised these for each recording. As a result, we were able to determine that delta and theta activity were clearly separable into two distinct frequency bands (delta 0.2-2Hz and theta 2.5-4.5Hz) (see Figure 2.8 below).

Once we had confirmed our desired frequency bands, we determined how their power altered with time across the full recording period. In order to address this, we conducted spectrogram analysis on each recording, allowing us to assess the spectral power as a function of time (see Figure 2.9 below). We then took this spectral analysis and plotted the power of our desired bands as a percentage of total power over time. This allowed us to determine periods that were in a dominant state. The requirements for a section of the recording to be characterised as a particular dominant state were; 1) the power of the frequency band was 30% greater than that of the next band during theta, and 20% greater than the band below for delta and, 2) the dominant period had a duration of at least 3 seconds. These thresholds we determined by systematically increasing the state separation requirements in increments of 10%, ranging from 10 to 40%. During this analysis, we found that applying a 20% dominance requirement for delta resulted in optimum loss of the theta component, whilst retaining the delta component which reduced in quality at 30, and 40% dominance thresholds. For theta separation, a 30% threshold was applied as this resulted in optimum separation of the theta component while also removing as much of delta component as possible. At 40% dominance, the quality/power of the theta component was reduced (see Figure 2.10). As a result, the LFPs were separated into theta-dominant, delta-dominant or an unclassified state when the requirements were not met. We then ranked these time periods in order to assess the period over which activity was defined as being within each of these state-dominant periods.
Figure 2.8. Illustration of LFP and FFT pre- and post state separation.
Figure illustrates that multiple rhythmic oscillations are contained within the duration of the recording. (A) Raw LFP over the entire recording (B). Theta dominant LFP signal and the respective FFT (right). (C) Delta/SW dominant LFP signal and the respective FFT (right). Note that whilst these periods represent a ‘dominant’ state other frequencies are still present, albeit at a lower power than that for the dominant frequency. Note that the FFTs to the right of the figure are representative of the entire theta and delta-dominant period, repectively. The raw state-dominant traces to the left of these show a representative portion of the signal.
Figure 2.9. Determination of state-dominant periods in LFP signal. (A) An example of a spectrogram, illustrating the spectral power across the whole recording. There are distinct oscillations between periods showing predominantly slower or higher frequencies under urethane anaesthesia. (B) The spectral power of delta, theta, beta and gamma rhythm LFP bands over time. Note that in order to be classed as delta/theta dominant, the spectral power had to be 20/30% greater than the next most powerful signal (power split=% of total power). (C) Theta/delta dominant event periods are then ranked and saved for further analysis (acc duration: accumulated duration).
Figure 2.10. State separation based on the relative dominance versus other rhythms. Example FFTs demonstrate how state separation was performed based on the relative dominance of the rhythm of interest versus other rhythms. Top row shows the difference in the resulting FFT following a requirement to be 10, 20, 30, and 40% stronger than the next underlying signal for delta rhythms. Note that a 20% separation results in optimal separation, with a 30 and 40% threshold resulting in poor signal quality. The bottom row shows the resulting FFTs following the same thresholds for theta rhythms. Note that a 30% difference results in optimal power separation without compromising the quality of the resulting FFT as seen when a 40% separation was applied.
2.9. Data analysis

2.9.6 Power analysis

In order to assess age- and disease-related alterations in the power of network oscillations, we conducted power analysis based on FFTs over the entire recording period.

Since our LFP recordings were obtained using MEAs spanning a dorso-ventral and proximo-distal axes of CA1 and subiculum, we decided that it was most appropriate to conduct all further analysis based on the average responses from all individual contacts. This would help reveal average functional/power changes from each region, and is more translatable to a non-invasive clinical assessment such as EEG. It would be unfeasible to place recording electrodes into regions of a human brain unless for clinical reasons (e.g., diagnosis of epileptic foci), therefore, using average values is more representative of non-invasive EEG recordings.

FFT analysis was conducted for each animal at each age and genotype and then averaged within regions. In detail, FFT analysis was conducted for each individual electrode located within each region, and then averaged within the region to produce one area-specific average FFT for each animal. We took this decision as individual FFT plots were similar, and therefore averaging across individual electrodes would remove any potential for bias through selection of specific channels to analyse further. An example of individual channels and mean FFTs is illustrated in Figure 2.11. Using the average FFT, we separated the power within each band of interest (delta 0.2-2Hz, theta 2.5-4.5Hz, alpha 9-16Hz, beta 16-30Hz, slow-wave ripples 120-180Hz and gamma 30-100Hz). We then calculated average power within each band and compared this within and between genotype and across age using two-way ANOVA, followed by post-hoc Bonferroni analysis.

2.9.7 Transfer entropy analysis

To date, the majority of research has focused on the assessment of functional connectivity between regions of the brain through the use of correlation and power analysis. However, these methods are unable to determine true causality between these signals, that is, whether one signal has an influence over another and vice versa. In addition, these methods are unable to discount the effect of any common input to both regions that may, to some extent, control the relationship of the LFP between the recorded regions of interest.

In order to address this issue and to assess the functional relationship between the signals obtained in CA1 and subiculum under spontaneous activity, we made use of transfer entropy (TE) analysis \cite{Schreiber2000}.
Figure 2.11. Example of individual and region-specific average FFTs. Example FFT plots for 11 channels located within the subiculum of a 3 month control mouse (black plots). It is evident that each channel, irrespective of specific anatomical location, has a similar FFT profile, and therefore it is appropriate to average across channels. The resulting average FFT plot from these 11 individual channels is shown in blue.
2.9. Data analysis

Information theoretic approaches were first introduced due to the need to quantify the fidelity of information passing through a communications system (Shannon 1948). The calculation of $TE$ combines the calculation of entropy values from a discrete variable (the LFP) with causality principles (Granger 1969) and techniques obtained from information theory (Schreiber 2000). This allows us to calculate a reliable measure of causality between linear and non-linear signals.

In order to calculate causality between two signals, firstly we must compute their Shannon entropy values. The Shannon entropy is typically defined as the average ‘unpredictability’ of a given variable in the signal/response. To calculate the entropy $H(X)$ of variable $X$ we use:

$$H(X) = - \sum_x P(x) \log_2 P(x)$$

(2.2)

Where $x$ represents all possible states of $X$ and $P(x)$ represents the probability of observing that particular state of $X$.

Following the calculation of the entropy values, we then developed this further and calculated the mutual information ($MI$) shared between the two signals. This builds upon Shannon entropy by determining how much one signal informs us about the other, i.e., how much signal $X$ informs us about signal $Y$. This can be thought as a reduction in uncertainty of signal $X$ by knowing a value from signal $Y$. As such, $MI$ determines how much information is shared between the signals and is calculated as the difference between the summed individual entropies $H(X)$ and $H(Y)$ and the joint entropy $H(X,Y)$.

$$H(X,Y) = - \sum_x P(x,y) \log_2 P(x,y)$$

(2.3)

$$MI(X,Y) = H(X) + H(Y) - H(X,Y)$$

(2.4)

Where $H(X,Y)$ is the conditioned entropies between $X$ and $Y$, and $P(x,y)$ is the probability of observing a particular state of $x$ at the same time as $y$.

In order to quantify the causal relationships between two signals, we must implement $TE$ analysis. This calculation allows us to determine how informative the source signal (e.g., signal $Y$), is for the past values observed in the target signal (e.g., signal $X$) over a range of intervals (tau). This can be calculated in both directions and, therefore, allows us to calculate how much one signal predicts the future state of another. In detail, $TE$ calculates a causal influence of signal $Y$ on signal $X$ through a reduction of uncertainty of the present value of $X$ by observed the past value of $Y$. In order to remove the influence of the past values of signal $X$, the current value of signal $X$ is conditioned to the past values of $X$. Therefore, if we obtain a
positive value, then we can show that having the previous history of signal Y is more informative than have the past history of signal X alone. As mentioned previously, we can then introduce a delay (tau), allowing for us to determine how informative one signal is over another over a given period. The calculation used in this study is below:

\[
TE = H(X_{t+\tau} | X_t) - H(X_{t+\tau} | X_t, Y_t) \\
= H(X_{t+\tau}, X_t) - H(X_t) - H(X_{t+\tau}, X_t, Y_t) + H(X_t, Y_t)
\] (2.5)

\[
TE = H(X_{t+\tau}, X_t) - H(X_t, Y_t)
\] (2.6)

\(TE\) can determine causality between two signals in both linear and non-linear systems whilst discounting for any affect due to a common/shared input. Therefore, the use of \(TE\) has many benefits over the use of coherence analysis and causality alone (Granger, 1969; Schreiber, 2000; Besserve et al., 2010; Vicente et al., 2011).

Here, signals were analysed using custom designed Matlab scripts to assess the information carrying capacity of LFP signals between CA1 and subiculum. We determined how information flow between these regions changed as a function of delay (tau=0-1000ms in 5ms intervals) during delta and theta periods. As demonstrated previously, it can be difficult to separate out periods of delta and theta reliably, and when we do, there is clear contamination from other underlying oscillations (Figure 2.9). Therefore, we conducted analysis on both state dominant periods of the signal (filter-free), as well as for LFP values band-pass filtered for the relevant state. In addition, we determined the \(TE\) of each state based on the raw LFP, amplitude and phase to determine which components of the LFP signal had the greater information carrying capacity.

We calculated the \(TE\) values for each of the conditions above and between each electrode pairs. The delay parameter (tau) was changed systematically in 5ms intervals to assess the extent to which the past of signal Y exerted a causative effect on signal X. Upon completion of the \(TE\) calculations, average values were obtained within each region at each delay. Values were then averaged within genotypes and compared. As a result, this allowed for direct comparison between each genotype at each delay. In addition, we calculated differences in the information carrying capacity of signals based on the peak and mean information value across delays and comparing these across age and genotype. Age and group differences were determined through the application of two-way ANOVAs, and post-hoc LSD or Bonferroni analysis.


2.10 Image processing and cell counting

Nissl stained sections were processed and photographed using a slide scanning light microscope attached to image processing software (Microscope: Olympus BX41, Slide-scaner, Software: ImagePro, MediaCybernetics, USA). Regions of interest were marked and slide scanning initiated to photograph the required region. A number of images were obtained (3-4) for each age and genotype.

Using image processing software (ImagePro plus, MediaCybernetics, USA) subicular cell counts were obtained for 3-4 images per animal (6 animals per genotype and age) so that averages could be obtained. Firstly, images were converted to 8-bit grey scale and Nissl-positive cells detected by dark-contrast detection. The subicular region was then marked, and a cell count performed. This provided the total number of cells within the subiculum and the total area of the region of interest. Counts were then converted to cells/mm$^2$. Averages were calculated for each animal, followed by group and age averages and compared to the counterpart genotype. All data were processed and analysed using Prism 5 (GraphPad, UK).
Chapter 3

Paper 1

Characterisation of neuronal deficits in the CA1-subicular pathway of the 3xTgAD mouse \textit{in vivo}
Daniel Squirrell\textsuperscript{1}, Sarah Fox\textsuperscript{1}, Marcelo Montemurro\textsuperscript{1}, John Gigg\textsuperscript{1,*}
\textsuperscript{1} Faculty of Life Sciences, The University of Manchester, Manchester, UK
* E-mail: J.Gigg@manchester.ac.uk

3.1 Abstract

Alzheimer’s disease (AD) is a progressive neurodegenerative condition resulting in the gradual loss of cognitive function, including the formation and retrieval of declarative memories, and changes in personality. However, despite the importance of characterising prodromal AD, little research has focused on assessing the neuronal changes associated with the early stages of the disease, particularly \textit{in vivo}. Recent work has characterised a specific, age-dependent deficit for episodic-like memory in the 3xTgAD mouse model of AD. This form of memory is dependent on the hippocampus, one of the prime targets for pathological development in AD, and therefore we aimed to determine whether this cognitive deficit is associated with age- and pathological-dependent alterations in the dorsal hippocampal formation, specifically, the connection from CA1 to subiculum.

A bipolar stimulating electrode was placed into dorsal CA1 and a multi-electrode recording array into dorsal subiculum of urethane-anaesthetised, 3xTgAD and control mice from groups aged 3, 6 and 9-months old. In order to assess synaptic integrity, we measured subicular responses to a variety of single, paired and repetitive stimulus patterns delivered to CA1. Finally, brains were removed and subjected to histological
analyses to determine whether 3xTgAD mice suffer from cell loss within the subiculum, and to correlate synaptic changes with accumulation of Aβ1-42.

Our results show that: a) 3xTgAD mice display similar basal subicular synaptic responses to control mice; however, deficits in subicular responses to high-frequency stimulation are apparent. Specifically, 3 month old 3xTgAD mice display impaired short- and long-term synaptic potentiation which progressively deteriorate with age and pathological progression; and b) 3xTgAD mice show a reduction in neuronal facilitation during repetitive train stimuli. We demonstrate that these neuronal deficits are specific to the amplitude of the evoked response, with slope values remaining relatively intact. Therefore, this provides evidence for a local change in the inhibitory control of the subiculum in the 3xTgAD mouse.

These experiments provide the first evidence that neuronal communication between dorsal CA1 and subiculum is affected during the early stages of AD-like pathology in the 3xTgAD mouse. We hypothesise that these changes in 3xTgAD mice are due to up-regulation of the local subicular inhibitory network.

3.2 Introduction

Alzheimer’s disease (AD) is a neurodegenerative condition resulting in the progressive decline in cognitive function. The risk of developing AD is enhanced with age and results in a significant personal and societal burden. Transgenic mouse models overexpressing the human forms of the amyloid precursor protein (APP), presenilin (PS1/PS2) and tau are commonly utilized in order to develop our understanding of AD (Bilkei-Gorzo 2014; Hall and Roberson 2012; Oddo et al. 2003; Duff et al., 2014; Games et al., 1995; Chapman et al., 1999; Duff et al., 1996; Jacobsen et al., 2006; Fitzjohn et al., 2001; Gureviciene et al., 2004). Expression of human APP and/or PS1/PS2 results in overproduction of beta-amyloid (Aβ1-42) leading to a neurotoxic increase in intracellular Aβ and extracellular plaque production (Oddo et al., 2003; Mastrangelo and Bowers, 2008). Whilst only a minority of AD cases are found to have this direct genetic link (2-5%), the pathological phenotype of the disease captured by these models is common to all clinically diagnosed AD cases. Two hallmark pathological features are present in post-mortem brains of AD patients; Aβ plaques and intracellular neurofibrillary tangles (NFTs) (Braak and Braak, 1991, 1997). As a result, there must be common factors between all patients that lead to this pathological phenotype. As there is currently little understanding of the physiological changes in the early stages of AD, due to the difficulty in directly measuring neuronal changes in human patients, it is vital for us to advance our understanding through the use of well-established mouse models of the disease.
3.2. Introduction

The 3xTgAD mouse expresses the familial transgenes for human APP_{SWE}, PS1_{M146V} and tau_{P301L}. This leads to overproduction of A\beta and tau pathology targeted to the hippocampal formation (HF) and cortex in a spatial and temporal manner similar to that found in AD patients (Mastrangelo and Bowers, 2008). Studies have shown that this model develops cognitive deficits at a young age, with recent work indicating subtle changes in episodic memory at 3 months that progressively deteriorates to become behaviourally significant by 6 months (Davis et al., 2013b,a). 3xTgAD mice also show impaired basal synaptic transmission and long-term potentiation (LTP) by 6 months between hippocampal regions CA3 and CA1 in vitro (Oddo et al., 2003). Until recently there had been no in vivo electrophysiological recordings in the 3xTgAD mice and only a few other studies in different AD mouse models (Gureviciene et al., 2004; Gengler et al., 2010; Witton et al., 2014; Cheng and Ji, 2013; Scott et al., 2012). Recent work on the 3xTgAD mouse shows evidence of hyperexcitability in the dentate gyrus (DG) and CA1 upon in vivo stimulation of the perforant pathway (PP). This study shows an early effect of AD-like pathology in hippocampal network connectivity, specifically hyperactivity of the input layers at 4 months (Davis et al., 2014). However, to date, the majority of studies evaluating the impact of AD-like pathology on hippocampal function has been studied in the reduced slice preparation (Bampton et al., 1999; Chapman et al., 1999; Fitzjohn et al., 2001; Jacobsen et al., 2006). This experimental technique results in the loss of bidirectional cortical connectivity, which is an essential component required for normal memory function. The type and the age at which changes are observed seem to depend on both the experimental preparation, i.e., in vitro or in vivo and the regions assessed. As a result, it is clear that further investigation is required, particularly through in vivo assessment, of the hippocampal system in order to fully understand the physiological changes that occur due to AD progression.

Evidence suggests that AD related changes occur at a young age, prior to overt plaque pathology, and that intracellular A\beta may correlate with the onset of functional and cognitive deficits (Billings et al., 2005; Oddo et al., 2003; Davis et al., 2013b,a, 2014). However, there has been limited research on how this may affect neuronal physiology at the network level. Studies show that pathological changes first become apparent in the HF, specifically within the entorhinal cortex (EC), and the border between CA1 and subiculum (Mastrangelo and Bowers, 2008; Braak and Braak, 1997). Whilst the subiculum is recognised as both a pivotal integrator and output region for hippocampal processing, and is one of the first regions to be affected by AD pathology, it has still received comparatively little attention.

Here, we examined the functional connectivity within the HF, specifically between CA1 and subiculum for the first time in the 3xTgAD mouse in vivo. Specifically, we measured the synaptic integrity of the monosynaptic connections between CA1
and subiculum through the recording of evoked extracellular field potentials (EPSPs) in response to stimulation of the CA1 pyramidal cell layer (Gigg et al., 2000; Commins et al., 1998).

## 3.3 Materials and Methods

### 3.3.1 Animals

Experiments were conducted on male 3xTgAD and C57/129sv mice, where the original breeding pair were kindly donated by the LaFerla lab (University of California, USA) (Oddo et al., 2003). The 3xTgAD mouse is a murine model of AD carrying the human transgenes APP\textsubscript{SWE}, PS1\textsubscript{M146V} and Tau\textsubscript{P301L}. Briefly, to generate the model the human transgenes for APP\textsubscript{SWE} and Tau\textsubscript{P301L} were microinjected in single cell embryos of homozygous PS1\textsubscript{M146V} mice. The offspring were then backcrossed multiple times in order to create stable homozygous offspring carrying all three genes, resulting in the generation of 3xTgAD mice. The 3xTgAD colony was then maintained ‘in-house’ through pairing of homozygous individuals, followed by confirmation of genetic stability by genotyping subsets of mice to confirm the presence of the three genes. Control mice (C57/129sv) were of the background strain of the PS1 knock-in mouse, and therefore also that of the 3xTgAD mouse. Similarly to the 3xTgAD mice, control mice were housed in groups of 4-6 and maintained ‘in house’.

Mice were housed with same sex and genotype cage-mates of 4-6 individuals in a pathogen free environment on a 12hr light:dark cycle (lights on at 7am) with food and water available \textit{ab-libitum}. All experimental procedures were performed in accordance with the Animals (Scientific) procedures Act 1986 (UK).

Data was collected from mice aged 3-4, 6-7, and 9-10 months to ensure that we include both the early onset of cognitive deficits and intracellular A\textsubscript{\beta} and later progression to extracellular plaque formation (Billings et al., 2005; Davis et al., 2013b,a; Mastrangelo and Bowers, 2008).

### 3.3.2 Anaesthesia and surgery

Anaesthesia was induced via intraperitoneal (i.p) injection of urethane (1.5-1.7g/kg of 30% w/v solution prepared in 0.9% Saline, Sigma, UK) and monitored until areflexia was achieved. Where reflexes remained, additional anaesthetic was administered by means of a ‘top-up’ dose (50\mu l of 10% w/v urethane solution prepared in 0.9% Saline). Breathing and survival under urethane was aided by performing a tracheotomy
Core body temperature was maintained at $37^\circ C$ for the duration of the experiment through use of a rectal thermometer placed underneath the abdomen and a homeothermic blanket.

Mice were mounted and fixed in a stereotaxic frame (Kopf 1430, USA), comprising of ear and bite bars in order to immobilise the head prior to surgery. The head was orientated in a position to match the mouse brain atlas \cite{Franklin2007} to aid electrode placement.

A midline scalp incision was made and the scalp retracted to expose the skull. Co-ordinates for electrode placements were then marked relative to Bregma and the midline for dorsal CA1 (Bregma: -1.5mm, ML: 1.7mm) and subiculum (Bregma: -2.8mm, ML: 1.7mm). The distance between Bregma and Lambda was measured prior to marking of positions in order to compare to the distance according to the mouse brain atlas (4.2mm). Co-ordinates for craniotomies and subsequent electrode placement were then calculated proportionately according to these reference landmarks to improve accuracy of placements. Small craniotomies were then drilled at marked co-ordinates using a 0.9mm drill bit (Fine Science Tools, Germany) and a high-speed hand held drill (Foredom, USA).

A multi-electrode recording array (see below) was lowered into dorsal subiculum at a compound angle of $25^\circ$ (from vertical) to accommodate for the curvature of the subiculum to match with the main dendritic orientation of subicular pyramidal cells; see Figure 3.1. The electrode array consisted of thirty two $413\mu m^2$ contacts spanning over two shanks (16 per shank) with $100\mu m$ vertical spacing between contacts and $500\mu m$ horizontal spacing between shanks (A2x16-10-100-500-413, NeuroNexusTech, USA) (see Figure 3.1). Recording electrodes were coated in Vibrant®CM-DiI (Life Technologies, UK) cell-labelling solution prior to insertion to allow for visualisation of electrode placement through use of post-hoc fluorescence microscopy. So that we can assess the flow of information between CA1 and subiculum during spontaneous LFP activity, a CA1 recording electrode was inserted into the hippocampus, prior to insertion of a bipolar stimulating electrode to assess evoked responses (see below). Note that in this chapter we report the results obtained from subicular evoked responses. Results measured during CA1 and subiculum spontaneous recordings are presented in the next results chapter.

Upon completion of the spontaneous recording sessions, lesions were created by passing a positive current through two contacts on the CA1 electrode ($30\mu A$ for 6 seconds). This helps to determine the exact positioning of the electrodes during post-hoc assessment. Note that the production of these lesions has a minimal effect on the integrity of the network \cite{Townsend2002}. This electrode was then removed and replaced by a bipolar stimulating electrode. A bipolar stimulating
3.3. Materials and Methods

A twisted 125µm diameter Teflon-insulated stainless-steel wires; Advent RM, UK) was positioned at the surface of the cortex and then slowly lowered during stimulus delivery (see below) until the CA1 pyramidal cell layer was reached and a characteristic evoked laminar profiles was seen in subiculum (Figure 3.1).

3.3.3 Stimulation

Stimuli were delivered by a constant-current stimulator (DS3, Digitimer, UK), which was triggered by analogue 5V square wave pulses from a National Instruments PCI card (PCI-6071E). Stimulus patterns were controlled through custom designed programs written in LabVIEW (v8, National Instruments). Stimulus duration was fixed at 200µs throughout and inter-pulse interval was 5s.

We first confirmed that the recording electrode correctly spanned the cell and dendritic layer of the dorsal subiculum. This was achieved through stimulation of the CA1 pyramidal cell layer and monitoring for the presence of a stable fEPSP in the subiculum using a 300µA positive current. A typical extracellular subicular evoked LFP consisted of an initial negative event (presumed EPSP; latency to trough ∼5ms), followed by a positive event (presumed IPSP). Reversal of the initial LFP was evident on contacts positioned further towards the tip of the shank, that is, towards the hippocampal fissure, together with a slightly delayed negative event, presumably caused by back-propagation into the dendrites (e.g., Leung and Peloquin (2006)). In all experiments, we successfully managed to evoke a stable subicular response profile, indicating that both the stimulating and recording electrodes were correctly positioned (see Figure 3.1).

Once a stable characteristic laminar response profile was achieved along the full axis of the electrode an input-output (I/O) curve was plotted by application of low-frequency stimulation (LFS) over a range of current intensities (0.33Hz, 20 repeats at each of 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450 or 500µA). An average response negative amplitude value was calculated and the current required to elicit half the maximum response was set and applied for all subsequent stimulation (typically 150-200µA).

A paired-pulse protocol was utilised in order to probe the network’s ability to express short-term synaptic facilitation. Paired-pulses were delivered at various inter-pulse intervals (20, 50, 100, 200, 500 and 1000ms) with 20 repeats for each interval. Facilitation could be identified by an increase in the response to the second pulse (P2) compared to the first (P1).

To assess the additive effects of repeated stimulation in terms of synaptic
plasticity and re-entrance of activity around the hippocampal circuitry, a train of low-frequency stimuli was applied (20 stimuli at 5 or 10Hz with 5 repeats). These frequencies were chosen so that a comparison could be made against those used during paired-pulse stimulation (PPS) and were predicted not to result in any long-lasting changes in the network circuitry (Kloosterman et al., 2003, 2004). LFP responses were analysed further in response to P1, P5, P10 and P20.

We further assessed the ability of the neuronal connections between CA1 and subiculum to demonstrate evidence of LTP by means of a high-frequency stimulation (HFS) protocol. A baseline recording was taken, prior to application of the HFS, through application of 50ms PPS (30s ISI) for a period of 15 minutes. An HFS (200Hz burst of 20 pulses, with 5 repeats separated by 2s) was then applied to CA1, followed by a series of 50ms PPS for a period of 60 minutes post HFS (120 repeats, 30s ISI).

Upon completion of all stimulation protocols, mice were transcardially perfused with 0.9% Saline Nitrate solution followed by 4% paraformaldehyde and brains removed for fixation. Tissue was then processed and section at 50\(\mu\)m on a sagittal plane and subjected to a standard Nissl staining procedure. Through the combination of visualising the tissue under a light (Olympus, BX41) and fluorescence microscope (Olympus, BX51) electrode tracks could be detected.

### 3.3.4 Data acquisition and analysis

Signals were recorded using a Recorder64 system (Plexon Inc, USA) with reference to a common ground and amplified through an AC-coupled headstage of a fixed x20 gain and further amplified to a total gain of x500. LFP signals were sampled at 2KHz and a low-pass filter of 250Hz applied using a 12-bit A/D convertor.

Raw fEPSP response amplitudes to single pulse stimulation were plotted to display their current-response relationship, constructing an I/O curve. Paired pulse and train data were normalized to the first pulse within animals (within the train) and then represented as a percentage change to the first response for amplitude and slope values from the fEPSP. This allowed us to determine the ability of CA1→subicular fibres to potentiate their responses. Train data were also subjected to further current source density (CSD) analyses to assess the functional connectivity between these regions (see below). In order to assess the ability of synapses to undergo LTP, responses during the baseline stimulation period were normalized to their average response value. Responses to the post-HFS low frequency stimulation train were expressed as a percentage change to the pre-HFS baseline. Results were then compared to a hypothetical value of 100% (i.e. no potentiation) using a two-tailed
one-sample t-test.

In detail, all paired-pulse responses were normalised to the first pulse and calculated by: 
\[ ((P2-P1)/P1) \times 100 \]

Responses to HFS were calculated by normalising the post-HFS responses (HFS2) to the pre-HFS baseline response (HFS1), and presented so that 100% represented no change: 
\[ (((HFS2-HFS1)/HFS1) \times 100) + 100 \]

All numerical data were analyzed using Prism 5 (GraphPad, UK). Two-way ANOVA followed by Bonferroni post-hoc comparisons were applied to identify any pair-wise genotype difference in each of the measures. Note that LTP induction was evaluated by a two-tailed one-sample t test, where the mean post-HFS response was compared to a hypothetical value of 100% (i.e. to facilitation).

### 3.3.5 Current source density analysis

Current source density analysis of extracellular recordings from laminar structures, such as that in the subiculum, provides a highly detailed spatiotemporal map of current dynamics produced through synaptic activity. Responses to P1, P5, P10 and P20 from the stimulation train from each of the 5 and 10Hz dataset were subjected to 1D CSD analysis by estimating the second order spatial derivative of their laminar field potential profiles (see equation 3.1) \cite{Nicholson1975}.

\[ CSD(h,t) = \frac{\sigma(h)(\Phi(h - n\Delta h, t) - 2\Phi(h, t) + \Phi(h + n\Delta h, t))}{(n\Delta h)^2} \]  

\[ CSD\ (h,t) \] is the CSD at a fixed time (t) and depth (h), \( \sigma(h) \) is the tissue conductivity (assumed to be constant), \( \Phi(k,t) \) is the field potential at a given time and depth, \( \Delta h \) is the distance between the contacts on the electrodes (100\ \mu m) and \( n \) is the smoothing value. The smoothing value (n) was set to ‘4’ to generate a smooth CSD profile. Current source density values were generated (presented here as arbitrary units) and scaled to maximum/minimum values within each animal through P1-P20 measurements. Current sources were illustrated in red, sinks in blue and neutral regions in green. Measurements were then recorded from each of these characteristic CSD events from each animal at each age and genotype in order to investigate if there was a change in these components as a function of age and/or AD-like pathology.
3.3.6 Image processing and cell counting

Nissl- and Aβ$_{1-42}$ staining sections were processed and photographed using a slide scanning light microscope attached to image processing software (Microscope: Olympus BX41, Slide-scanner, Software: ImagePro, MediaCybernetics, USA). Regions of interest were marked and slide scanning initiated to photograph the required region. A number of images were obtained (3-4) for each age and genotype.

Using image processing software (ImagePro plus, MediaCybernetics, USA) subicular cell counts were obtained for 3-4 images per animal (6 animals per genotype and age) so that averages could be obtained. Firstly, images were converted to 8-bit grey scale and Nissl-positive cells detected by dark-contrast detection. The subicular region was then marked, and a cell count performed. This provided the total number of cells within the subiculum and the total area of the region of interest. Counts were then converted to cells/mm$^2$. Averages were calculated for each animal, followed by group and age averages and compared to the counterpart genotype. All data were processed and analysed using Prism 5 (GraphPad, UK).

3.4 Results

We successfully recorded from 3-4 month (control n=9, 3xTgAD n=8), 6-7 month (control n=9, 3xTgAD n=10) and 9-10 month (control n=10, 3xTgAD n=9) mice. Electrode positions have been confirmed for all data presented within the results below.

3.4.1 Similar response profiles between all control and 3xTgAD mice

For the first time we are able to evoke and report the structure of subicular responses following CA1 stimulation in mice. We show that there is a stable and characteristic subicular response profile, representing the summation of extracellular dendritic activity (Figure 3.1B). This characteristic response is dominated by a negative going EPSP, increasing with depth towards the hippocampal fissure. We also note the response becomes slightly delayed with depth, likely due to back-propagation through the apical dendrites of subicular cells (Leung and Peloquin, 2006).
3.4. Results

Figure 3.1. Subicular evoked response profiles are similar between control and 3xTgAD mice. (A) HF with positions of the stimulating (red) and 32-channel multi-electrode array (blue) marked. Stimulation within the CA1 pyramidal cell layer generates a characteristic response profile along the dorsal-ventral axis of subiculum (A: right). (B) Average EPSP laminar response profiles from control (black) and 3xTgAD (blue) mice at 3 months. Responses show a very stable and comparable response profile between genotypes.

3.4.2 Changes in basal synaptic connectivity

In order to detect any changes in basal synaptic connectivity between CA1 and subiculum, we applied a range of currents to evoke and measure subicular EPSPs. We measured both slope and amplitude values from subicular evoked EPSPs, to determine their input-output relationship following CA1 stimulation.

Two-way (genotype and intensity) ANOVAs, for both slope and amplitude measurements, show that stimulation intensity had a significant effect on both slope and amplitude measurements for 3 month (slope: F(11,174)=3.76, p<0.0001, amplitude: F(11,174)=3.98, p<0.0001), 6 month (slope: F(11,186)=11.38, p<0.0001, and amplitude: F(11,186)=17.84, p<0.0001) and 9 month (slope: F(11,126)=6.24, p<0.0001, and amplitude: F(11,126)=7.59, p<0.0001) mice (Figure 3.2). We found no genotypic difference at 3-4 months in any of the measurements assessed in the two-way ANOVAs (Figure 3.2), suggesting that these mice are born without deficits in basal connectivity between CA1 and subiculum (Figure 3.2). Whilst mice are born functionally intact, we did see changes in basal synaptic connectivity as a function of AD-like pathology progression. Genotypic differences first become apparent by 6 months, when compared to age-matched control mice (F(1,186)=5.19, p<0.05) for amplitude measurements only; responses did not significantly differ when assessed based on response slope. A genotypic amplitude-specific deficit in 3xTgAD mice
was also apparent at 9 months (F(1,126)=5.65, p<0.05), however, it is worth noting
that at 6 months 3xTgAD mice had a greater response than that in the control mice
with the opposite observed at 9 months. Therefore, complex differences are observed
which may indicate different stages of disease-like progression.

Changes in neuronal connectivity were seen to alter as a function of age only. Thus, two-way (genotype and age) ANOVAs indicated that basal connectivity de-
creased in both genotypes as function of age, between 3 and 9 months in both
slope (control F(2,255)=11.97, p<0.0001, 3xTgAD F(2,231)=15.08, p<0.0001) and
amplitude measurements (control F(2,255)=9.96, p<0.0001, 3xTgAD F(2,231)=8.68,
p<0.001).

Whilst we were able to detect overall genotypic differences apparent by 6 months,
we were unable to detect any pair-wise differences following the application of a pair-
wise Bonferroni post-hoc test. Therefore, we propose that the genotypic difference in
basal connectivity is only mildly affected due to AD-like pathology.

3.4.3 Paired-pulse facilitation deficits are apparent from 3 months in
the 3xTgAD mouse

In order to assess the ability of the system to express paired-pulse facilitation (PPF),
we applied paired-pulse stimuli (range 20-1000ms ISI) using currents that elicited half-
maximal responses as determined through the to I/O curves. We then determined
the change in slope and amplitude measurements to the second pulse. All responses
were normalised to that of the first pulse to reflect a percentage change. A positive
value represents facilitation of the second response, and a negative value represents
synaptic depression. We determined the effect of paired-pulse interval and compared
between genotypes and age. All data were analysed through application of two-way
(interval and genotype) ANOVA, followed by post-hoc Bonferroni analysis.

To assess the impact of AD-like pathology on PPF we applied two-way (genotype
and interval) ANOVAs to measurements obtained for both slope and amplitude
values. Our results showed a clear effect of interval on both measurements at 3 month
(slope: F(5,90)=15.59, p<0.0001, amplitude: F(5,90)=18.16, p<0.0001), 6 month
(slope: F(5,102)=20.59, p<0.0001, and amplitude: F(5,102)=40.09, p<0.0001) and 9
month (slope: F(5,102)=19.02, p<0.0001, and amplitude: F(5,102)=24.43, p<0.0001)
mice (Figure 3.3). Additionally, the results demonstrate clear evidence of synaptic
dysfunction between genotypes in response amplitude in 3 month old 3xTgAD mice
F(1,90)=23.32, p<0.0001). Post-hoc Bonferroni tests detected pair-wise differences
at 20, 50 and 100ms intervals, whilst no overall or pair-wise differences were detected
in slope values (Figure 3.3). By 6 months we found similar PPF genotypic deficits
Figure 3.2. Current input-output response relationship. Stimulus response (I/O) curves for 3-, 6- and 9 month control (blue) and 3xTgAD (red) mice. Top row shows the I/O relationship for slope measurements, and bottom row shows this relationship for amplitude measurements. All comparisons were made by 2-way (genotype and intensity) ANOVA followed by post-hoc Bonferroni to reveal any pair-wise differences. Errors are shown as ±SEM.
in amplitude measurements (F(1,102)=15.45, p<0.001), with pair-wise differences
detectable at 20, 50 and 100ms intervals. However, we also report overall genotypic
and pair-wise (50ms) differences in slope values in 6 month mice (F(1,102)=15.45,
p<0.001). By 9 months of age we are unable to detect overall genotypic or pair-wise
differences in both slope and amplitude measurements. Therefore, by this age, control
mice have dropped to similar levels of facilitation as that within 3xTgAD mice.

In addition to changes in short-term synaptic performance with AD-like pathology
progression, we demonstrate an overall affect of age in control mice. Within
group two-way (age and interval) ANOVAs for slope and amplitude measurements
show that there are changes in synaptic plasticity associated with age in control mice
in both slope (F(2,150)=5.27, p<0.01) and amplitude (F(2,150)=14.72, p<0.0001).
Within group (age and interval) analysis for 3xTgAD mice fails to detect an age
related change in PPF. Therefore, the results showed that short-term plasticity is
affected early during AD-like pathology (from 3 months), whereas control mice show
a reduction in facilitation as a function of age, and by 9 months control mice drop to
similar levels to that observed during the early stages in 3xTgAD mice.

3.4.4 Differences in facilitation are not due to a change in the rate of
ascending phase of the evoked EPSP

To assess for the possible influence of a change in the levels of inhibition, we measured
the rate of change (slope) of the ascending phase of the evoked EPSP. We chose to
determine if there was a change in the ascending phase of the EPSP during 50ms
PPS. This would provide an indication into whether feedback/feedforward inhibition
is up regulated in the 3xTgAD mouse. In a similar way to previous measures, two
data points were chosen on the steepest part of the ascending phase of the upward
slope from each waveform, and slope values calculated. Averages were then calculated
within each animal and normalised to those obtained from the first pulse and plotted
as a percentage change. Two-way (genotype and age) ANOVA reveals that there is
no genotypic (F(1,47)=1.925, p=0.1719) or age-related (F(2,47)=2.488, p=0.0939)
effect (Figure 3.4).

3.4.5 Responses to stimulus trains

To determine the effect of repeated stimulation on neuronal reverberation, mice were
subjected to repeated low-frequency stimulation in the form of 20 pulses at 5Hz or
10Hz.
Figure 3.3. Early changes in short-term plasticity in the 3xTgAD mouse. PPF for 3, 6 and 9 month control (black) and 3xTgAD (white) mice. Top row shows the responses to PPF for slope measurements, and the bottom row shows responses to PPF for amplitude measurements. Genotype differences are most noticeable in amplitude facilitation. Inset illustrates an example of PPF in the voltage traces. Note that the dashed trace (response to the 2nd pulse) is larger than that to the first pulse (solid trace). All data were analysed using a two-way (genotype and interval) ANOVA followed by application of a Bonferroni post-hoc (*p<0.05, **p<0.01, ***p<0.001). Errors are ±SEM.
3.4. Results

Figure 3.4. No differences are apparent in the relative speed of the ascending phase of the evoked EPSP during 50ms PPS. Left image shows an example of two responses, one to first pulse and another to the second. Crosses indicate example positions at which data points were obtained and slope values calculated. Right shows that no differences were apparent in the relative speed of the ascending phase of the evoked EPSP during 50ms PPS. Facilitation of the ascending phase of the evoked EPSP during 50ms PPS is illustrated, with facilitation represented as a percentage change in response to values recorded during the first pulse. No genotypic or age-related changes were observed in these measures. All data were analysed using a two-way (genotype and age) ANOVA. Errors are ±SEM.

Changes in neuronal activity to 5Hz train stimulation

Repeated stimulation at 5Hz resulted generally in synaptic facilitation with apparent deficits in 3xTgAD mice as young as 3 months. Following two-way (genotype and pulse number) ANOVAs for both slope and amplitude measurements, we showed that during each train, synaptic facilitation increased as a factor of pulse number for both slope (Figure 3.5A) and amplitude in both groups (Figure 3.5B) at 3 months (slope: F(19, 320)=12, p<0.0001, and amplitude: F(19,320)=11.41, p<0.0001), 6 months (slope: F(19,320)=8.34, p<0.0001, and amplitude: F(19,320)=7.43, p<0.0001) and 9 months (slope: F(19,280)=7.67, p<0.0001, and amplitude: F(19,280)=10.54, p<0.0001). Whilst pulse number had an effect on facilitation within genotype and age for slope and amplitude, we also found an effect of genotype. We find evidence of early synaptic dysfunction in 3- and 9 month 3xTgAD mice. Our results indicated that there are genotype differences in amplitude measurements in 3 month (F(1,320)=95.68, p<0.0001) and 9 month mice (F(1,280)=104.29, p<0.0001), post-hoc tests revealing pair-wise differences at pulse 15, 16 and 18-20 at 3 months and 19-20 at 9 months. Within group two-way (age and pulse number) ANOVAs for both slope and amplitude measurements showed a general decrease in amplitude facilitation with age during trains in control mice (F(2,480)=142.97, p<0.0001), with similar changes for both slope (F(2,440)=10.13, p<0.0001) and amplitude (F(2,440)=38.17, p<0.0001) for 3xTgAD mice. As such, there is a clear early deficit
3.4. Results

in neuronal facilitation to low-frequency stimulation at 3 months in 3xTgAD mice. Levels of facilitation decrease with age in both genotypes, however, this age- and AD-like pathology-related decrease is lost at 6 months. By 9 months we find further evidence of an age- and disease-related reduction in facilitation. It is therefore conceivable that different mechanisms are attributable to the age-related differences observed at 3 and 9 months (Figure 5A and B). We also found very limited evidence of synaptic reverberation (‘re-entrance’) during the earlier pulses in the stimulation train, with evidence of potential reverberation becoming apparent during the later stages in subiculum in both genotypes for 5 and 10Hz trains.

Changes in neuronal activity to 10Hz train stimulation

As per the 5Hz trains, repeated stimulation at 10Hz resulted in synaptic facilitation and apparent synaptic deficits in 3xTgAD mice as young as 3 months. Following the application of two-way (genotype and pulse number) ANOVAs for both slope and amplitude measurements, our data indicated a significant effect of pulse number, that is, synaptic facilitation increased during the 10Hz train, for both slope (Figure 3.5C) and amplitude (Figure 3.5D) at 3 months (slope: F(19, 320)=6.75, p<0.0001, and amplitude: F(19,320)=7.03, p<0.0001), 6 months (slope: F(19,320)=7.14, p<0.0001, and amplitude: F(19,320)=3.2, p<0.0001) and 9 months (slope: F(19,280)=4.06, p<0.0001, and amplitude: F(19,280)=7.25, p<0.0001). There was also an effect of both genotype and age for both slope and amplitude, with evidence of early synaptic dysfunction in 3- and 9 month 3xTgAD mice. Our results indicate that that there are genotypic differences in amplitude and slope measurements in 3 month (slope: F(1,320)=25.23, p<0.0001, amplitude: F(1,320)=50.32, p<0.0001) and amplitude values in 9 month mice (F(1,280)=139.42, p<0.0001). Post-hoc pair-wise assessment revealed genotypic differences in amplitude measurements at 9 months at pulse numbers 14-20. Two-way (age and pulse number) ANOVAs for both slope and amplitude measurements detected a general decrease in facilitation during the stimulus train with age for both slope (F(2,480)=4.81, p<0.01) and amplitude (F(2,480)=19.66, p<0.0001) for control mice and slope (F(2,440)=24.80, p<0.0001) and amplitude (F(2,440)=20.52, p<0.0001) for 3xTgAD mice. As such, there is an early deficit in neuronal facilitation to low-frequency stimulation at 3 months in 3xTgAD mice. Similarly to the results observed during the 5Hz stimulation trains, we found an age- and AD-related reduction in facilitation in both genotypes that is apparent at 3 and 9-months, with a lack of an observable change at 6 months (Figure 3.5C and D). Unlike the large synaptic reverberation observed within DG following PP stimulation (Davis et al 2014), we found limited evidence of synaptic reverberation (‘re-entrance’) into subiculum in either genotype during 10Hz stimulation, with some
potential evidence becoming apparent during the later stages of the train when evaluated using CSD analysis.

3.4.6 Current source density to 5Hz low-frequency stimulation reveals intact CA1→subiculum connectivity

The spatial arrangement of CA1→subicular synaptic current sinks and sources are comparable between genotypes during 5Hz CA1 repetitive stimulation. Control and 3xTgAD mice showed evidence of a strong sink-source pair within the cell layer (component 1) that was maximal approximately 5-6ms following CA1 stimulation and reversed in the molecular layer (component 2). This most likely reflected initial subicular activation to CA1 stimulation (Figure 3.6A). There was a later sink-source pair (components 3 and 4) in control and 3xTgAD mice immediately following subicular activation, most likely reflecting local inhibition following initial activation. These results support the view that CA1 inputs terminate in the subicular cell layer, resulting in the generation of the earliest subicular current sink (component 1). This then generates a dipole, resulting in a current source located within the subicular dendritic layer (component 2).

This sink-source combination (components 1 and 2) was visible throughout each stimulus train. This suggests that the CA1→subicular input relationship is maintained and unaffected through repetitive stimulation. We also report that the location and maintenance of these dipoles are comparable between control and 3xTgAD mice (Figure 6A). In addition, there was late (∼15ms) synaptic activity in the subicular dendritic layer by P20 in control mice, with a noticeably smaller source in the 3xTgAD mice. This may reflect entorhinal re-entrance through neuronal reverberation (late current sources are marked *).

To determine any changes in the strength of the sink and sources located within the subiculum, we measured the strength of each of the CSD components during pulse 1 and 10. Two-way ANOVA (genotype and component) revealed a lack of genotypic differences at any age in any component. Further, our analysis of responses to the first pulse (P1) failed to reveal any genotypic differences in any component at any age. Therefore, both I/O and CSD analyses demonstrate a lack of functional changes in basal synaptic transmission in the 3xTgAD mouse when compared with age-matched control mice (Figure 3.6).

1note that responses to the first pulse combined those measured during the 5Hz and 10Hz stimulation train
### 3.4. Results

<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Slope</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Hz stimulation</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
</tr>
<tr>
<td>10 Hz stimulation</td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
</tr>
<tr>
<td><strong>Amplitude</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Hz stimulation</td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
<td><img src="image9" alt="Graph" /></td>
</tr>
<tr>
<td>10 Hz stimulation</td>
<td><img src="image10" alt="Graph" /></td>
<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
</tr>
</tbody>
</table>

Figure 3.5. Low-frequency repetitive stimulation reveals synaptic deficits in 3xTgAD mice during the early stages of AD-like pathology. Subicular responses to repeated low-frequency stimulation of CA1 at 5Hz (top) and 10Hz (bottom) in control (blue) and 3xTgAD (mice) aged 3, 6 or 9 months (left, middle and right). Data represent evoked EPSP slope (A and C) and amplitude (B and D). All responses were normalised to those of the first pulse and expressed as a percentage change in relation to the first response. Therefore positive values represent synaptic facilitation and a negative value would represent synaptic depression. All data were analysed by two-way (genotype and pulse) ANOVA, followed by Bonferroni post-hoc test for comparisons (*p<0.05, **p<0.01, ***p<0.001). Errors are ±SEM.
3.4. Results

Figure 3.6. Current source density analysis of responses during 5Hz train. Average 3-month CSD profiles of subicular responses to CA1 stimulation are morphologically similar between control (A top) and 3xTgAD (A bottom) throughout the stimulation train (P1, P5, P10 and P20). There are four stable and reproducible components, most likely representing direct CA1→subicular EPSP sink (1) and source (2), followed by subicular inhibition (3 and 4). We also detect a late current source in control and 3xTgAD mice, which is most likely due to re-entrance of neuronal activity through entorhinal reverberation (*). Peak strengths of CSD components 1-4 during pulse 1 (left) and pulse 10 (right) are presented for 3 (B), 6 (C) and 9 month old mice (D). All data were analysed by two-way (genotype and component) ANOVA, followed by Bonferroni post-hoc test for comparisons (*p<0.05, **p<0.01, ***p<0.001). Errors are ±SEM.
3.4.7 Current source density to 10Hz low-frequency stimulation reveals intact CA1→subiculum connectivity

Similar to 5Hz stimulation, the positions of CA1→subicular sinks/sources were comparable between genotypes during a 10Hz CA1 repetitive stimulation. Again, control and 3xTgAD mice show evidence of a strong sink-source pair approximately 5-6ms following CA1 stimulation, most likely reflecting initial subicular activation to CA1 stimulation (components 1 and 2) (Figure 3.7A), with a later common sink-source pair in both control and 3xTgAD mice. This mostly likely reflects inhibition of the subicular response following initial activation (components 3 and 4). The spatial arrangement of sink-source pairs is qualitatively similar to that seen with 5Hz stimulation (compare Figure 3.5A to Figure 3.6A).

We also observed late (~15ms) synaptic activity in the distal subicular dendritic layer by P20 in control mice, which was noticeably later (~20ms) in the 3xTgAD mice (late current sources are marked *). This may reflect entorhinal re-entrance through neuronal reverberation.

In order to determine any changes in the strengths of the sink and sources located within the subiculum, we measured the strength of each of the CSD components during pulse 1 and 10. The 10Hz stimulation allowed us to assess the effect of repetitive stimulation with 100ms inter-stimulus interval. By pulse 10, we saw evidence of potential network reverberation in some of the individual voltage plots, and therefore justifies further evaluation by CSD analysis. As a result, and due to time constraints we did not assess the strength of CSD components at pulse 20. Our data showed that there is no evidence of changes in CSD components during 10Hz stimulation (Figure 3.7B).

3.4.8 3xTgAD mice are unable to express long-term potentiation

Recent work has indicated that the CA1→subiculum pathway in the rat can express LTP in vivo, and that LTP is depressed in vitro in the CA3-CA1 pathway in the 3xTgAD mouse (Commins et al., 1998; Oddo et al., 2003). To gain a greater understanding of the neuronal changes associated with age and AD-like pathology, we assessed the ability of the CA1 input to subiculum in our mice to express long-term changes in synaptic weights.

Whilst we were unable to obtain stable baseline recordings for 9 month-old control and 3xTgAD mice, we were able to successfully induce significant LTP that persisted for at least 1 hour in the subicular region of control mice for both slope \( t(9)=3.647, p<0.01, \text{mean: } 127.8\% \) and amplitude \( t(9)=2.351, p<0.05, \text{mean:} \)
Figure 3.7. Current source density analysis of subicular responses to 10Hz stimulation of CA1. Average 3-month CSD profiles of subicular responses to CA1 stimulation are morphologically similar between control (A top) and 3xTgAD (A bottom) throughout the CA1 stimulation train (P1, P5, P10 and P20). As per 5Hz stimulation, there were four stable and reproducible components, most likely representing CA1→subicular sink (1) and source (2), followed by subicular inhibition (3 and 4). Peak strengths of each CSD components during pulse 1 (left) and pulse 10 (right) are presented for both genotypes aged 3 (B), 6 (C) or 9 months old (D). The late current source in control and 3xTgAD mice most likely reflects re-entrance of neuronal activity through entorhinal reverberation (*). All data were analysed by two-way (genotype and component) ANOVA, followed by Bonferroni post-hoc test for comparisons (*p<0.05, **p<0.01, ***p<0.001). Errors are ±SEM.
3.4. Results

119.1% at 3 months, and slope (t(7)=2.894, p<0.05, mean: 115.6%) and amplitude (t(7)=2.930, p<0.05, mean: 113.2%) at 6 months (Figure 3.8). 3xTgAD mice were unable to express LTP for slope or amplitude at either age. Therefore, we detect an early (3 month) and persistent deficit in LTP induction in 3xTgAD mice when compared to age-matched controls.

![Example control mouse](Image1)

![Example 3xTgAD mouse](Image2)

Figure 3.8. Early deficits in LTP induction in the 3xTgAD mouse. (A) Example of successful LTP induction, with baseline stimulation represented in blue, and post-HFS stimulation in red. Note that responses to LFS post-HFS are elevated compared to those recorded prior to HFS. (B) Example of unsuccessful LTP induction in 3xTgAD mice. Post-HFS responses are similar to those recorded prior to HFS. Successful induction of LTP is evident in slope (C) and amplitude (D) measurements in both 3- and 6-month controls but not 3xTgAD mice. All data were analysed by a one-sampled t-test (compared to 100% baseline values) (*p<0.05, **p<0.01). Errors are ±SEM.

3.4.9 No neuronal cell loss with age or AD-like pathology progression

We investigated whether the alterations in neuronal communication could be due to a direct result of neuronal cell loss. We demonstrate that there is no age- or AD-like pathology related gross degeneration in subicular neurons (Figure 3.9). Two-way (age and genotype) ANOVAs reveal no significant change in overall neuron numbers within the subiculum.
3.5 Discussion

To date there has been limited investigation into the neuronal changes associated with AD-like pathology progression in the 3xTgAD mouse, with only one *in vivo* and *in vitro* study (Davis et al., 2014; Oddo et al., 2003). This therefore limits our understanding of AD mechanisms and what impact AD-like pathology has on the synaptic integrity within many sub-regions of the HF in the intact mouse (i.e. *in vivo*). The CA1 and subiculum are two of the keys regions of the HF to show early and severe pathological changes in both human AD patients and mouse models of the disease. Whilst these regions are traditionally considered to be the hippocampal output regions, surprisingly few studies have assessed how pathology in this pathway so central to the impact of AD affects neuronal function. Here, we report an *in vivo* assessment of neuronal function in this comparatively under-investigated CA1-subiculum pathway in urethane-anaesthetised mice. This study is one of only a few to investigate the neuronal changes associated with age and AD-like pathology progression in AD mouse models *in vivo* (Oddo et al., 2003; Davis et al., 2014).

Firstly we investigated the synaptic integrity of basal neuronal communication between CA1 and subiculum. To determine the level of basal connectivity between CA1 and subiculum, we stimulated CA1 with increasing current intensities and constructed an I/O curve. Basal connectivity was intact in the 3xTgAD mouse at 3 months, confirming that the 3xTgAD mouse is born without functional neuronal changes (Oddo et al., 2003; Davis et al., 2014). However, at both 6 and 9 months, we detected genotype differences in basal transmission, with 3xTgAD mouse displaying altered I/O curves. This is in agreement with data from some (Fitzjohn et al., 2001; Hsia et al., 1999; Oddo et al., 2003) but not all similar studies in various AD models,
3.5. Discussion

albeit in different regions to those studies here (Davis et al., 2014; Chapman et al., 1999). In particular, we report that these I/O differences for CA1-subiculum are more pronounced in the amplitude rather than the slope of evoked subicular EPSPs. This suggests that CA1 input fibres to subiculum remain functionally intact, with no clear difference in the ability of subicular afferents to induce excitatory responses. However, although the rate of depolarization in CA1-induced subicular activation is similar (as shown by similar slope measures) the amplitude of depolarisation is decreased in 3xTgAD compared to control mice. Therefore, we propose that subicular input fibres remain intact and, therefore, any functional neuronal changes are not due to changes in basal connectivity or neuronal conductance (Desai et al., 2009).

In order to examine short-term plasticity, mice were subjected to a paired-pulse paradigm that is known to induce PPF in the rat CA1-subicular pathway (Commins et al., 1998). In support of other studies, we report that these connections are able to express short-term plasticity in response to PPS (20-1000ms). We demonstrate that PPS results in increased facilitation at intervals between 20-100ms, similar to that reported in the rat subicular pathway (Commins et al., 1998). In support of other studies in the 3xTgAD and other mouse models of AD, we report that there are early detectable changes in neuronal plasticity (Davis et al., 2014; Jacobsen et al., 2006; Gengler et al., 2010). However, whilst Davis et al. (2014) demonstrated hyperexcitability in the DG and CA1 of 3xTgAD mice in response to PP stimulation, we show the CA1-subicular pathway shows early hypoexcitability over the same age range in these mice. Thus, we detected an early and significant reduction in facilitation to short intervals (20-100ms) at both 3 and 6 months in evoked EPSP measurements.

In addition to an early genotype difference in short-term plasticity, we find that synaptic function alters with age. Specifically, control mice show an age-related decrease in PPF by 9 months towards 3xTgAD levels, supporting the conclusion that 3xTgAD mice undergo an advanced ageing-like phenotype. This is similar to our previous findings (Davis et al., 2014) and agrees with other research that there is a decrease in neural excitability with age, most likely due to increased inhibitory after-potentials and accommodation to responses (Disterhoft and Oh, 2007).

In order to further assess the general excitability of the HF, we applied a high frequency repetitive LFS to CA1 pyramidal cells. Other studies have shown that similar stimulation paradigms are capable of inducing hippocampal reverberation through re-entrant EC excitatory activity feeding back into the hippocampus (Kloosterman et al., 2003, 2004; Davis et al., 2014). However, the ability of subiculum to support reverberatory activity has not been tested previously. This reverberation of neuronal activity through the hippocampal-EC-hippocampal system, is impor-
tant for strengthening of circuits, and to allow for the comparison of old and new information. Recent work in the 3xTgAD mouse demonstrated that EC PP input fibres appear functionally intact in both young and old AD mice, and that young (4-6 month) 3xTgAD mice were hyperexcitable following application of 5- and 10Hz repetitive stimuli with evidence of neuronal reverberation [Davis et al., 2014]. Davis et al. (2014) demonstrated that there was an overall reduction in facilitation to train stimuli in both DG and CA1 regions of the hippocampus in 3xTgAD mice. In addition, they also demonstrated that 3xTgAD mice had a greater propensity to generate a population spike in the DG to the first pulse of a stimulus pair, indicating increased excitability in young mice (Davis et al., 2014). With age and AD-like pathology progression, the authors found that there was a reduction in facilitation and a genotype difference becoming apparent in aged 3xTgAD mice. This is in support of our train data in the subiculum. We find that 3xTgAD mice show a genotype reduction in amplitude facilitation as young as 3 months, which continues to be apparent at 9 months. We also show that there is a general reduction in facilitation of evoked EPSP amplitudes as a function of age, but that EPSP slope values remain unaffected. This is most likely due changes in inhibition, and likely age-related increased inhibitory after-potentials and neuronal accommodation [Davis et al., 2014; Palop et al., 2007; Disterhoft and Oh, 2007]. Interestingly, in order to help determine the contribution of feedback/feedforward inhibition, we measured the upward ascending phase of the evoked EPSP during 50ms PPS (Figure 3.5). Whilst these results indicate that is no direct change in the relative speed of the ascending phase of the evoked EPSP, it does not necessarily rule out a change in the inhibitory tone in the 3xTgAD mouse. It is indeed possible that, whilst we find no effect in this particular measure, the inhibitory mechanisms in the 3xTgAD mouse may be more sensitive, and therefore be recruited earlier during the descending phase of the EPSP. However, we are unable to determine the exact mechanistic changes in inhibition and whether or not this is due to changes in feed-forward, such as that found in DG (Davis et al., 2014), or feedback inhibition.

We also report limited evidence of substantial re-entrance present within the subiculum following CA1 stimulation in both control and 3xTgAD mice. This is in stark comparison to original findings in the rat, and compared to our previous findings that showed substantial re-entrance through the EC via the PP, with 3xTgAD mice displaying greater levels of neuronal reverberation [Davis et al., 2014; Kloosterman et al., 2003, 2004]. We also report a slight increase in latency in the presentation of hippocampal re-entrance within the 3xTgAD groups. Our results indicate that AD-like pathology has little impact on neuronal reverberation through subiculum, and is likely to be due to an overall up-regulation in inhibition within the subiculum. This is in contrast to recent findings reported by Davis et al. (2014) who show an
increase in excitability and subsequent neuronal re-entrance within DG and CA1. Entorhinal cortex layer III fibres terminate within CA1 slm and subiculum molecular layers, with recent work indicating that there is strong neuronal reverberation within the CA1 slm region (Davis et al., 2014; Kloosterman et al., 2003, 2004). However, our data shows little baseline and AD-like pathology related alterations within the subiculum. Therefore, our results also show a difference in the regulation of the EC layer III input to CA1 and subiculum. We know that the DG is relatively unaffected by AD pathology and that the EC and subiculum are some of the first brain regions to become affected by pathology in both human patients and mouse models of AD (Braak and Braak, 1991; Mastrangelo and Bowers, 2008). In addition, it is known that subicular activation leads to complex activation of 1) the direct pathway from EC to DG, and 2) a complex multi-synaptic pathway to CA1 or re-entrance from the deep to superficial layers of EC. Davis et al. (2014) report early changes in the PP, with a presumed reduction in feedforward inhibition, leading to synaptic hyperexcitability. In contrast, we demonstrate for the first time that whilst reverberation/re-entrance is clearly demonstrated in the system, the reduction in excitability between CA1 and subiculum will lead to an overall reduction in the propagation of network excitability. Our results show an early, AD-like pathology related down-regulation in neuronal excitability, and therefore are indicative of a functional disconnect between CA1 and subiculum, which may help to explain the onset of cognitive differences as early of 3 months in the 3xTgAD mice (Davis et al., 2013b,a).

In terms of the spatio-temporal pattern of evoked synaptic currents in subiculum these also agree with our previous findings in DG/CA1, that is, the location and strength of the sink/sources remains intact with age and AD-like pathology. We show that CA1 inputs produce a dipole within the cell layer of subiculum, as demonstrated by the current sink, with a concurrent source in the dendritic layer. This appears to back-propagate into the molecular layer in both groups, the first evidence for such a physiological phenomenon in the subiculum. We also show that there is a later sink/source pair, most likely reflecting recurrent inhibition within the cell layer that ‘reverses’ in the moleculare (Menendez de la Prida and Gal, 2004).

In order to assess the impact of AD-like pathology on the ability of subicular CA1 inputs to express long-term synaptic potentiation, we applied a high-frequency stimulation protocol previously shown to induce LTP in the CA1-subicular pathway in rats (Commins et al., 1998; Craig and Commins, 2006). Here, we demonstrate for the first time, that that this projection also supports LTP in mice however, to a much lower degree than that reported in rats in vivo. In addition, we show that there
is an early dysfunction in 3xTgAD mice, with an inability to induce LTP within the subiculum. This finding is in support of other studies in the same and other models of AD within different projection systems (Oddo et al., 2003; Gureviciene et al., 2004; Jacobsen et al., 2006; Gengler et al., 2010). Our data provides the first evidence of neuronal plasticity existing between CA1 and subiculum in the mouse. We report evidence of deficits in the neuronal plasticity during the early stages of AD-like pathology in the 3xTgAD that increases in severity as a function of age and pathology progression. Down-regulation in PPF and LTP are likely to have a direct phenotypic effect, leading to alterations in short- and long-term memory consolidation and retention. Although the hippocampal network is a complex and highly inter-connected region, our results correlate with the onset and progression of alterations in cognitive capacity. Recent work has shown the first detectable changes in cognition as early as 3 months, with subtle alterations during episodic-like hippocampal-dependant tasks (Davis et al., 2013b,a), and deficits in long-term retention at 4 months (Billings et al., 2005).

Synaptic dysfunction is central to AD and its progression. Here we show that there is a clear deficit in neuronal transmission between CA1 and subiculum prior to overt AD-related pathology (Oddo et al., 2003; Davis et al., 2014; Hsia et al., 1999). Importantly, our work correlates well with the onset and development of cognitive deficits in these mice. Studies have demonstrated that the 3xTgAD mouse has a selective hippocampal-dependent episodic-like memory deficit at 6 months, with evidence of above chance but decreased performance at 3 months (Davis et al., 2013b,a). Studies also show that the 3xTgAD mouse has hippocampal spatial deficits at 4-6 months (Billings et al., 2005), correlating with the development of intracellular Aβ oligomers (Billings et al., 2005; Oddo et al., 2003; Davis et al., 2014). Whilst we were unable to quantify levels of Aβ in the subiculum, we do report that intracellular Aβ_{1-42} was present as early as 3 months, prior to overt plaque production (data not shown). Aβ oligomers are known to play a key role in synaptic dysfunction in AD. Studies have indicated that application of oligomers induces morphological and neurotransmitter changes, which ultimately result in synaptic depression (Lambert et al., 1998; Hsia et al., 1999; Hardy and Selkoe, 2002; Kamenetz et al., 2003). Recent work further demonstrated the toxic effect of Aβ oligomers with application of oligomers to hippocampal slices resulting in reduced amplitude of extracellular mini excitatory postsynaptic potentials, a loss of AMPA receptors and reduction in their opening probability with a subsequent loss of dendritic spines (Parameshwaran et al., 2007; Yamin et al., 2009; D’Amelio et al., 2011; Li et al., 2009).

Our results support other work that suggests that a variety of cognitive and neuronal deficits develop and correlate with the detection of intracellular oligomeric forms of Aβ, and that cognitive and neuronal changes can and do appear prior to
Aβ plaques (Mastrangelo and Bowers, 2008; Oddo et al., 2003; Billings et al., 2005; Davis et al., 2013b; Hsia et al., 1999).

We propose that the changes observed in the subicular region of the 3xTgAD mouse are most likely due to network changes in excitability. These changes are likely to be due to a combination of factors, including AD-related pathology, and changes due to an advanced ageing phenotype. AD pathology and age can result in the reduction of AMPA receptor excitability and even a decrease in receptor numbers, resulting in an overall reduction in glutamatergic transmission (Isaac et al., 1995). Interestingly, we only detected genotype differences in the amplitude responses of evoked EPSPs, with intact slope values. As such, we propose that there is an early up-regulation of inhibitory tone within the CA1-subiculum pathway by 3 months in the 3xTgAD mouse. This increased inhibition also seems to be a normal process of ageing, and that AD mice experience an advanced ageing phenotype in terms of subicular function.

In addition to network changes in levels of inhibition, other work has indicated possible changes in calcium handling as a mechanism resulting in the change in levels of excitation. Since PPF relies on changes in calcium levels in the presynaptic terminal (Zucker and Regehr, 2002), changes in calcium handling are likely to alter neurotransmitter release probability at synapses. Whilst some studies have indicated that the introduction of the APP transgene negates the effect on calcium handling due to the PS1 transgene (Oddo et al., 2003), other studies have shown that calcium related changes in the 3xTgAD mouse remain (Smith et al., 2005; Lopez et al., 2008). However, we find that there is a slight rightward and downward shift in the I/O curve in the 3xTgAD mouse, and therefore it is unlikely that there is an elevated release of calcium in the presynaptic terminal. As a result we conclude that it is unlikely that changes in calcium handling contribute to the synaptic failure found in this study.

In support of our conclusions, Davis et al. (2014) suggested that there was a reduction in feed-forward inhibition in the DG and CA1, with increased late feedback inhibition and inhibitory after-potentials. Other studies have implicated a role of astrocytes in the GABAergic control of neuronal function within the hippocampus in AD. Resting levels of calcium are increased in astrocytes of AD mice, leading to increased calcium waves and subsequent release of GABA (Jo et al., 2014). Increased GABA release is found to result in an increased GABAergic tone, inhibiting synaptic release in the hippocampus. To further support our theory that the 3xTgAD mouse expresses an advanced ageing phenotype, we show that neuronal excitability decreases as a function of age. We show that by 9 months facilitation levels are at similar levels in both genotypes, and that this correlates with behavioural studies indicating
that both control and AD mice are impaired in episodic-like memory tasks at this age (Davis et al. 2013b,a).

In summary, we demonstrate for the first time the presence of early deficits in CA1-subiculum neuronal connectivity in the 3xTgAD mouse model for AD. These deficits are present by 3 months of age and broadly result in synaptic hypoexcitability. Whilst other in vivo studies have indicated the earliest detectable changes in neuronal connectivity present as hyper-excitability, particularly in DG and CA1 (Davis et al. 2014), we find evidence of synaptic hypo-excitability prior to stages of hyperexcitability observed in other regions of the HF. Since the border between CA1 and subiculum, and the entirety of the subiculum region are one of the first regions be affected by AD-like pathology, we propose that these differences observed in the onset and development of synaptic alterations are likely to be contributed to the underlying pathological state of the brain. Recent work supports these findings, where researchers found evidence for pathology-related changes in neuronal excitability, with Aβ accumulation initially producing a state of hyperexcitability which then progresses to state of synaptic hypo-excitability (Palop and Mucke 2010b,a). The EC and subiculum are the principal regions of the HC to be affected by early AD pathology (Braak and Braak 1991, 1997; Mastrangelo and Bowers, 2008) and, therefore, changes in synaptic function are likely to occur earlier within these regions when compared to others. When combined with our previous study on DG and CA1, the present findings suggest that age and AD-like pathology related changes across the HC occur at different developmental stages of AD-like pathology and possibly different rates in the 3xTgAD mouse. In particular, the age range over which we see subicular hypo-excitability in the present findings overlaps with that at which we see hyper-excitability in DG and CA1. As such, it is important for future assessment of neuronal function and development of therapeutics for AD to consider the implications of these complex network changes.
Chapter 4

Paper 2

An in vivo assessment of the information carrying capacity of the CA1-subicular pathway in the 3xTgAD mouse
Daniel Squirrel\textsuperscript{1}, Maria Constantinou\textsuperscript{1}, Marcelo Montemurro\textsuperscript{1}, John Gigg\textsuperscript{1,*}
1 Faculty of Life Sciences, The University of Manchester, Manchester, UK
* E-mail: J.Gigg@manchester.ac.uk

4.1 Abstract

Alzheimer’s disease (AD) is a neurodegenerative condition resulting in the gradual decline in cognition; including a loss in the formation and retrieval of declarative memory, sleep disturbances, and changes in personality. Whilst AD is a debilitating condition, posing a significant emotional and financial stress to the patient, carers and family members, there is still a lack of understanding of the early neuronal changes during the prodromal stages of the disease. It is widely accepted that neuronal changes occur prior to alterations in pathology and cognition, and therefore it is vital for us to develop our understanding of the network alterations during these stages. It is also important for us to develop techniques that allow detection of quantifiable changes in biomarkers during the early prodromal stages of AD.

Here, we aimed to determine the impact of AD-like pathology on the information carrying capacity of the monosynaptic connections between dorsal CA1 and subiculum. These regions display pronounced pathology at early stages of AD in both patients and AD models. Multi-electrode recording arrays were placed into dorsal CA1 and subiculum in the urethane anaesthetised 3xTgAD mouse model of AD and age-matched control mice. Groups were assessed at 3, 6 or 9 months old. We recorded a
range of local field potential (LFP) activity, including theta and delta oscillations. Through the application of novel information theoretic techniques we quantified the information carrying capacity of the network during different network oscillatory states.

Our results show evidence of a significant and early breakdown in the information carrying capacity of the CA1-subiculum network. This is apparent as early as 3 months in the 3xTgAD mice during periods of delta dominant activity, with a specific deficit in the slow-wave ripple component of the signal. This change may underlie the documented deficit in memory consolidation at this age in 3xTgAD mice. We also show that changes in the information carrying capacity are complex, and are age- and AD-like pathology dependent, with later changes observed in the theta and slow-wave components of the LFP signal. In particular, 3xTgAD mice show a breakdown in the information carrying capacity of the network during periods of theta activity by 6 months, and in the slow-wave component of the delta rhythm by 9 months.

The present data show for the first time evidence of measureable network changes in information transfer capacity that directly correlates with the onset of cognitive and pathological deficits in the 3xTgAD mouse. This data, therefore, may provide a physiological explanation for the early memory retention and concurrent sleep disturbances observed in AD patients. Therefore, it is likely that the application of information theoretic techniques will provide us with a useful biomarker for the detection and characterisation of hippocampal network changes during the prodromal stages of the disease.

4.2 Introduction

Alzheimer’s disease (AD) is a neurodegenerative condition and the most common form of dementia, resulting in the progressive loss of cognition and changes in personality. AD is commonly associated with changes in intra-regional synaptic connectivity and alterations in network activity (Palop and Mucke, 2010b,a; Rubio et al., 2012), along with concurrent changes in the connectivity between inter-connected cortical regions (Kosik, 2013; Jeong et al., 2001).

Changes in network oscillations and inter-regional coherence have been studied for many years as a potential biomarker for diagnosing AD (Brenner et al., 1988; Erkinjuntti et al., 1988; Johannesson et al., 1979; Kaszniaik et al., 1979; Rae-Grant et al., 1987; Soininen et al., 1982; Jeong et al., 2001). Studies utilising cortical electroencephalogram (EEG) recordings have traditionally indicated that there is a
4.2. Introduction

A general slowing of rhythms and a net reduction in coherence between brain regions (Jeong, 2004). In addition to slowing in the peak frequency of the rhythms, research has shown that in AD there is a tendency for increased delta and theta power, and decreased alpha and beta power; in addition, these network changes correlate well with the severity of cognitive impairment (Brenner et al., 1988; Coben et al., 1983, 1985; Giaquinto and Nolte, 1986; Rae-Grant et al., 1987; Soininen et al., 1982; Kowalski et al., 2001). Therefore, there is substantial promise that recording brain rhythms can detect neuronal network changes in AD prior to clinical presentation of cognitive impairments (Jeong, 2004; Amieva et al., 2008).

However, whilst network activity obtained via recording the EEG is relatively cheap, widely available and non-invasive, it most frequently reflects cortical activity and, therefore, may be more useful for the detection of cortical rather than sub-cortical dementia (Verma et al., 1987; Jeong et al., 2001; Jeong, 2004). The correlation of a reduction in synaptic connectivity with alterations in cognition, supports the use of EEG recordings to help develop our understanding of the mechanisms of network changes in AD (Cook and Leuchter, 1996).

Whilst power and correlation analyses of cortical EEG activity provide an insight into how network activity may alter with age and disease progression, there has been a lack of studies that have aimed to quantify the flow of information between memory-relevant regions of the brain in AD, such as the hippocampal formation (HF). Advances in the assessment of relationships between brain regions have been made through the implementation of statistical and mathematical concepts, such as through the use of Granger causality and coherence analysis (Granger, 1969). However, traditional measures of functional connectivity, such as coherence and Granger causality are unable to account for alterations in statistical and mathematical relationships between two, or more signals in non-linear systems, such as those in EEG signals, and for intrinsically generated causalities due to common inputs (Friston, 2011; Schreiber, 2000; Granger, 1969). Therefore, in order to determine causality and to determine the information carrying capacity of a signal, it is important to build on these measures by introducing transfer entropy (TE) (Schreiber, 2000; Shannon, 1948).

In this study we make use of the 3xTgAD mouse, which is a well developed and characterised model of AD, expressing and developing AD-related pathology in a temporal and spatial manner similar to that found in human forms of AD, with CA1 and subiculum being two of the first regions to become affected by AD-like pathology (Oddo et al., 2003; Mastrangelo and Bowers, 2008; Braak and Braak, 1991, 1997). In addition, the 3xTgAD mouse has detectable changes in neuronal function at 4 months, impairments in associative memory at 3-4 months and deficits in spatial
navigation and episodic-like memory by 6 months (Oddo et al., 2003; Davis et al., 2013b,a, 2014; Billings et al., 2005). Therefore, this model not only develops the pathological phenotype of human AD, but also shows AD-like changes in neuronal function and cognitive deficits prior to the development of overt pathological lesions (Mastrangelo and Bowers, 2008).

This study, for the first time, aims to characterise the network changes associated with age and disease in the sub-cortical regions of the AD brain. Specifically, we focus on the final and comparatively under-investigated connection between CA1 and subiculum. For the first time, we apply a combination of Shannon entropy and information theory (Shannon, 1948; Schreiber, 2000) in order to determine the information carrying capacity of the CA1→subiculum pathway and how this alters with age and disease. In addition, we distinguish which features of the network activity contribute to this process.

4.3 Materials and Methods

4.3.1 Animals

3xTgAD mice carrying the human transgenes APP(SWE), PS1(M146V) and Tau(P301L) and age-matched non-littermate controls were bred at the University of Manchester from a colony originally donated by the LaFerla group (Oddo et al., 2003). Mice were housed in same sex and genotype cage-mates of 4-6 individuals in a pathogen free environment on a 12hr light:dark cycle (lights on at 7am) with food and water available ab-libitum. All experimental procedures were performed in accordance with the Animals (Scientific) procedures Act 1986 (UK). Data were collected from control and 3xTgAD male mice aged 3-4 months (n=7 and 8, respectively), 6-7 months (n=6 and 6, respectively) and 9-10 months (n=6 and 5, respectively). These ages were specifically chosen to coincide with the onset of cognitive deficits and intracellular Aβ and subsequent development of pathological lesions (Billings et al., 2005; Davis et al., 2013b,a; Mastrangelo and Bowers, 2008).

4.3.2 Anaesthesia and surgery

Anaesthesia was induced via intraperitoneal (i.p) injection of urethane (1.5-1.7g/kg of 30% w/v solution prepared in 0.9% Saline, Sigma, UK) and monitored until areflexia was achieved. Where reflexes remained, additional anaesthetic was administered by means of a ‘top-up’ dose (50µl of 10% w/v urethane solution prepared in 0.9% Saline). Breathing and survival under urethane was aided by performing a tracheotomy
Core body temperature was maintained at 37°C for the duration of the experiment.

Mice were mounted and fixed in a stereotaxic frame with mouse adapter (Kopf 1430, USA) prior to surgery. The head was orientated in a position relative to the mouse brain atlas (Franklin and Paxinos, 2007) to aid with electrode placement. A midline incision was made and the scalp retracted to expose the skull. Co-ordinates for electrode placements were marked relative to Bregma and skull midline for CA1 (Bregma: -1.5mm, ML: 1.7mm) and subiculum (Bregma: -2.8mm, ML: 1.7mm). The distance between Bregma and Lambda was measured prior to marking of positions in order to compare to the distance according to the mouse brain atlas (4.2mm). Where required, co-ordinates for craniotomies were adjusted proportionately according to these reference landmarks to improve accuracy of placements. Craniotomies were then drilled using a 0.9mm drill bit (Fine Science Tools, Germany) and a high-speed hand held drill (Foredom, USA).

Multi-electrode recording arrays were inserted into CA1 and subiculum, targeted to span the cell layers of each region. Arrays consisted of thirty two 413µm2 contacts spanning over two shanks (16 per shank) with 100µm vertical spacing between contacts and 500µm horizontal spacing between shanks (A2x16-10-100-500-413, NeuroNexusTech, USA). The CA1 recording electrode was inserted at a compound angle of 20° from vertical and lowered by 2.25mm from the surface of the cortex. The subiculum electrode was inserted at a compound angle of 25° (from vertical) and lowered by 2.38mm. The compound angle was chosen to allow for easy access of both electrodes and to accommodate for the curvature of the subiculum (so that the vertical axis of the electrode was matched with the main dendritic orientation of subicular pyramidal cells (see Figure 4.1). Recording electrodes were coated in Vibrant®CM-Dil cell-labelling solution (Life Technologies, UK) to allow for visualisation of electrode placement by post-hoc fluorescence microscopy.

### 4.3.3 Spontaneous recordings and data acquisition

Prior to recording, electrodes were left to settle for approximately 30 minutes. Spontaneous LFP activity was then recorded for a period of at least thirty minutes from each contact on both CA1 and subiculum electrodes simultaneously. Data were recorded on a Recorder64 system (Plexon Inc, USA) with reference to a common ground. Signals were amplified first through an AC-coupled headstage of fixed x20 gain and then a variable-gain amplifier, typically set to provide a total gain of x2000. LFP signals were sampled at 2KHz on each channel with a low-pass filter of 250Hz applied and digitized with a 12-bit A/D convertor. All data were recorded and saved.
for offline analysis.

During the recording session, activity from each individual contact could be visualised in more detail by monitoring through a series of oscilloscopes (HAMEG Instruments GmbH, US). Field and spiking activity were typically monitored in order to optimise electrodes positioning in their targets.

### 4.3.4 Perfusion and storage of brain tissue

To aid the localisation of recording electrodes lesions were produced through the application of positive current at two contacts on the CA1 electrode (30µA for 6 seconds) ([Townsend et al.](#) 2002). Upon completion of electrophysiological recordings an overdose of urethane was administered and mice were transcardially perfused with 0.9% saline nitrate followed by 4% paraformaldehyde solution (PFA) to fix brain tissue. Brains were then removed from the skull and stored in 4% PFA for ~3 days prior to sectioning. For long-term storage of tissue, brains were cyroprotected by sinking them in a 30% sucrose solution prior to placing them in antifreeze solution and then stored at -20°C.

### 4.3.5 Electrode localisation

Fixed brains were washed in 1xPBS and sectioned on a freezing microtome (Leica, SM 2400) in the sagittal plane at 30µm. Sections were suspended in 1xPBS and then mounted onto charged slides (Thermo Scientific, Superfrost Plus) from distilled water before being left to dry in the dark overnight.

CM-DiI-stained tracks were visualised under a florescence light microscope (Olympus BX51) with a 4x objective and images captured using a camera (Coolsnap ES camera, Photometrics) through imaging software (MetaVue, Molecular Devices). CM-DiI solution was excited through the use of a Texas Red filter and images merged with a background image of the region of interest. Images were then processed and analysed (ImageJ).

In order to visualise electrode lesions, tissue was then further processed and subjected to a standard Nissl stain procedure. Slides were then cover-slipped using DPX and left to dry. Slides were later viewed under a light microscope (Olympus, BX-51) and photographed (Image-Pro Plus, Mediacy, UK).
4.3.6 Electrophysiological state filtering

Urethane anaesthesia induces a state of unconsciousness similar to that found during physiological sleep (Clement et al., 2008). As a result, we were able to distinguish between distinct periods that resembled either theta or delta/slow-wave sleep (SWS) oscillations in network activity. To assess the impact of AD-like pathology on network oscillations, we separated periods dominated by either theta or delta band activity from the spontaneous LFP activity. To determine the most appropriate frequency bands that best separated these we created fast Fourier transforms (FFTs) for each contact within CA1 and subiculum. FFT data from each channel, within each region, was averaged to produce a mean FFT for CA1 and subiculum. This allowed for two prominent and consistent peaks in power to be determined in each animal. As a result, we determined that a peak in power lying between 0.2-2Hz represented dominant delta activity and a peak between 2.5-4.5Hz dominant theta activity.

The duration of delta- and theta-dominant periods were determined using spectral analysis. We constructed a spectrogram for each recording point within each region and plotted delta and theta power as a percentage of total power for each sample point across the entire recording. As a result, we were able to separate periods of state-dominance based on the following criteria; 1) the power of the delta (0.2-2Hz) and theta (2.5-4.5Hz) frequency band was 20% (for delta) and 30% (for theta) greater than the second most powerful band, and 2) the dominant period had a duration of at least 3 seconds. State-dominant periods were then ranked in order to assess the duration over which activity was defined as being within each of these state-dominant periods.

4.3.7 Band-pass filtering for delta, theta and slow-wave ripple oscillations

In order to determine the information carrying capacity of the LFP during different oscillatory states, we applied band-pass filters (Matlab, Version 7.10, Mathworks, USA.) to separate periods of delta (0.2-2.5Hz), theta (2.5-4.5Hz) and slow-wave ripples (SWRs) (120-180Hz). This allowed us to perform analyses on specific oscillatory bands within each state-dominant period. This allowed for us to conduct further analyses on different band-specific oscillatory frequencies. Thus, we could determine the information carrying capacity of each individual frequency band, and therefore reveal their relative contributions in the raw/mixed signals.
4.3.8 Power analysis

FFT analyses were conducted for each animal at each age and genotype and then averaged within regions, that is, FFTs from one animal from subiculum recording electrodes were averaged to produce an average subiculum FFT. We decided to use average FFT data, as this allows for us to look at gross/regional changes and is more representative to the data obtained during more common human EEG recordings. Using the FFT, we separated the power within each band of interest (delta 0.2-2Hz, theta 2.5-4.5Hz, alpha 9-16Hz, beta 16-30Hz, SWRs 120-180Hz). We then calculated average power within each band and performed two-way (genotype and age) ANOVA, followed by post-hoc Bonferroni analysis.

4.3.9 Cross-covariance analysis

In order to determine how filtered delta and theta signals co-vary in relation to their means, we analysed these by cross-covariance at 3, 6 and 9 months in both genotypes. Whilst this analysis provides a useful means to demonstrate any relationship between two signals, and how this varies over time, it cannot determine causality between signals, shared information or discount for the influence of a common input. Therefore, we also subjected signals to transfer entropy (TE) analyses as detailed below.

4.3.10 Transfer entropy analysis

In order to determine causality between two signals, we firstly computed their Shannon entropy values. The Shannon entropy is typically defined as the average ‘unpredictability’ of a given variable in the signal/response. Entropy values are calculated based on the probability of observing particular variables within a signal, with greatest entropy values observed when all variables have equal probability. Probabilities were calculated through the application of a sliding window and binning of each window into 4 equal segments. This ensured that a probability distribution curve was produced for the entire portion of the signal of interest. To calculate the entropy $H(X)$ of variable $X$ we used the following;

$$H(X) = - \sum_x P(x) \log_2 P(x)$$

(4.1)

Where $x$ is the individual state of variable $X$ and $P(x)$ is the probability of observing a given state of $(x)$.

Following the calculation of the entropy values, we then calculated the mutual information (MI) shared between the two signals. MI builds upon Shannon entropy
by determining how much one signal informs us about the other, i.e., how much signal $X$ informs us about signal $Y$. This can be thought as a reduction in uncertainty of signal $X$ by knowing a value from signal $Y$. As such, $MI$ determines how much information is shared between the signals and is calculated as the difference between the summed individual entropies $H(X)$ and $H(Y)$ and the joint entropy $H(X,Y)$:

$$H(X,Y) = -\sum_x P(x,y) \log_2 P(x,y)$$  \hspace{1cm} (4.2)

$$MI(X,Y) = H(X) + H(Y) - H(X,Y)$$  \hspace{1cm} (4.3)

Where $H(X,Y)$ is the conditioned entropies between $X$ and $Y$, and $P(x,y)$ is the probability of observing a particular state of $x$ at the same time as $y$.

However, as $MI$ calculations are symmetric, that is $MI(X,Y) = MI(Y,X)$, it is unable to determine causality between one signal and another. In order to quantify the causal relationships between two signals, we implemented $TE$ analysis. $TE$ determines how informative a source signal (e.g. signal $Y$) is for the past values observed in a target signal (e.g. signal $X$) over a range of intervals ($\tau$). This can be calculated in both directions and, therefore, reveals how much one signal predicts the past state of another. In detail, $TE$ allows us to determine a causal affect of signal $Y$ on signal $X$ through the introduction of a temporal delay ($\tau$) to one of the signals. The temporal delay is implemented through the calculation of shared $MI$ between the present value of signal $X$ and past value of signal $Y$, conditioned to signal $X$ in the past. In effect, this allows us to determine whether or not having knowledge of the previous values of signal $Y$ allows for a greater predictability of signal $X$ in the present, above that of knowing previous values of $X$ alone. If this is the case, then we can infer that signal $Y$ must therefore have a causal effect on signal $X$. As mentioned previously, we can then analyse the effect of introducing a delay ($\tau$), allowing for us to determine how informative one signal is over another over a given period. The calculation used in this study is below:

$$TE = H(X_{t+\tau} | X_t) - H(X_{t+\tau} | X_t, Y_t)$$  \hspace{1cm} (4.4)

$$= H(X_{t+\tau}, X_t) - H(X_t) - H(X_{t+\tau}, X_t, Y_t) + H(X_t, Y_t)$$  \hspace{1cm} (4.5)

$TE$ allows for the determination of causality between two signals in both linear and non-linear systems whilst discounting for any affect due to a common/shared input. Therefore the use of $TE$ has many benefits over the use of coherence analysis and causality alone (Granger, 1969; Schreiber, 2000; Besserve et al., 2010; Vicente et al., 2011).

Here, signals were analysed using custom designed Matlab (R2013a, MathWorks,
4.4. Results

USA) scripts in order to assess the information carrying capacity of LFP signals between CA1 and subiculum. We determined how information flow between these regions changed, as a function of time (tau=0-1000ms in 5ms intervals). We conducted analyses on both theta- and delta-dominant periods of the signal and their band-pass filtered versions (for specific delta, theta and SWR bands). In addition, in order to determine which component of the LFP signal had the greatest information carrying capacity, we determined the $TE$ of each state based on the raw LFP, phase and amplitude components of the signal. Note that due to relatively low power of the alpha and beta bands in relation to other frequencies, we are unable to detect dominant periods of the LFP that occupy these spectra. Therefore, all $TE$ analyses were conducted on delta and theta components of the signal.

We calculated the $TE$ values for each of the conditions above and between each possible pairs of channels. The delay parameter (tau) was changed systematically in 5ms intervals ranging from 0-1000ms in order to assess the extent to which past values of signal Y exerted a causative affect on signal X. Upon completion of the $TE$ calculations, average values were obtained within each region at each delay. As a control, this procedure also incorporated a shuffling technique whereby signals were shuffled and would, therefore, lose their temporal coherence. In order to account for bias, $TE$ values obtained on shuffled data were then subtracted from those obtained from experimental LFPs. These differential values were then averaged within genotypes to provide a direct $TE$ comparison between each genotype at each delay. Based on this, we then investigated group differences by computing average $TE$ values across all values of tau and compared between genotypes. In addition, we calculated differences in the information carrying capacity of signals based on the peak and mean values across delays and compared these by application of two-way ANOVAs, and post-hoc LSD/Bonferroni analysis. For peak and mean information analysis, group differences were determined by two-way ANOVAs, followed by post-hoc Bonferroni tests. Due to the large number of comparisons within the two-way (genotype and time) ANOVAs (due to the large range for tau) we assessed effects of genotype and tau by pair-wise differences through the application of post-hoc LSD analyses.

4.4 Results

For the first time, we assessed the changes in network activity within the CA1-subiculum axis of the hippocampal formation, and how this is affected due to age and AD-like pathology progression. Specifically, we quantify changes in the information carrying capacity of LFP signals between CA1 and subiculum. We determined how information flow between these regions changed, as a function of time (tau=0-1000ms in 5ms intervals). We conducted analyses on both theta- and delta-dominant periods of the signal and their band-pass filtered versions (for specific delta, theta and SWR bands). In addition, in order to determine which component of the LFP signal had the greatest information carrying capacity, we determined the $TE$ of each state based on the raw LFP, phase and amplitude components of the signal. Note that due to relatively low power of the alpha and beta bands in relation to other frequencies, we are unable to detect dominant periods of the LFP that occupy these spectra. Therefore, all $TE$ analyses were conducted on delta and theta components of the signal.

We calculated the $TE$ values for each of the conditions above and between each possible pairs of channels. The delay parameter (tau) was changed systematically in 5ms intervals ranging from 0-1000ms in order to assess the extent to which past values of signal Y exerted a causative affect on signal X. Upon completion of the $TE$ calculations, average values were obtained within each region at each delay. As a control, this procedure also incorporated a shuffling technique whereby signals were shuffled and would, therefore, lose their temporal coherence. In order to account for bias, $TE$ values obtained on shuffled data were then subtracted from those obtained from experimental LFPs. These differential values were then averaged within genotypes to provide a direct $TE$ comparison between each genotype at each delay. Based on this, we then investigated group differences by computing average $TE$ values across all values of tau and compared between genotypes. In addition, we calculated differences in the information carrying capacity of signals based on the peak and mean values across delays and compared these by application of two-way ANOVAs, and post-hoc LSD/Bonferroni analysis. For peak and mean information analysis, group differences were determined by two-way ANOVAs, followed by post-hoc Bonferroni tests. Due to the large number of comparisons within the two-way (genotype and time) ANOVAs (due to the large range for tau) we assessed effects of genotype and tau by pair-wise differences through the application of post-hoc LSD analyses.

4.4 Results

For the first time, we assessed the changes in network activity within the CA1-subiculum axis of the hippocampal formation, and how this is affected due to age and AD-like pathology progression. Specifically, we quantify changes in the information carrying capacity of LFP signals between CA1 and subiculum. We determined how information flow between these regions changed, as a function of time (tau=0-1000ms in 5ms intervals). We conducted analyses on both theta- and delta-dominant periods of the signal and their band-pass filtered versions (for specific delta, theta and SWR bands). In addition, in order to determine which component of the LFP signal had the greatest information carrying capacity, we determined the $TE$ of each state based on the raw LFP, phase and amplitude components of the signal. Note that due to relatively low power of the alpha and beta bands in relation to other frequencies, we are unable to detect dominant periods of the LFP that occupy these spectra. Therefore, all $TE$ analyses were conducted on delta and theta components of the signal.

We calculated the $TE$ values for each of the conditions above and between each possible pairs of channels. The delay parameter (tau) was changed systematically in 5ms intervals ranging from 0-1000ms in order to assess the extent to which past values of signal Y exerted a causative affect on signal X. Upon completion of the $TE$ calculations, average values were obtained within each region at each delay. As a control, this procedure also incorporated a shuffling technique whereby signals were shuffled and would, therefore, lose their temporal coherence. In order to account for bias, $TE$ values obtained on shuffled data were then subtracted from those obtained from experimental LFPs. These differential values were then averaged within genotypes to provide a direct $TE$ comparison between each genotype at each delay. Based on this, we then investigated group differences by computing average $TE$ values across all values of tau and compared between genotypes. In addition, we calculated differences in the information carrying capacity of signals based on the peak and mean values across delays and compared these by application of two-way ANOVAs, and post-hoc LSD/Bonferroni analysis. For peak and mean information analysis, group differences were determined by two-way ANOVAs, followed by post-hoc Bonferroni tests. Due to the large number of comparisons within the two-way (genotype and time) ANOVAs (due to the large range for tau) we assessed effects of genotype and tau by pair-wise differences through the application of post-hoc LSD analyses.
4.4. Results

carrying capacity between CA1 and subiculum during periods of delta and theta activity. We recorded from 3xTgAD and aged-matched control (C57/129sv) mice aged 3 month (n=7 and n=8), 6 month (n=6 and n=6), and 9 month (n=6 and n=5).

4.4.1 Urethane anaesthesia induces reliable and stable sleep-like network oscillations

Urethane anaesthesia induced both delta- and theta-dominant network oscillations (see Figure 4.1). These oscillations, like those observed in physiological sleep, fluctuated during the recording, as clearly observed in the spectrogram analysis. Due to the relatively good signal:noise ratio and generation of two distinct bands in the FFTs, we could clearly distinguish periods when the spectrogram was dominated by either band (Figure 4.1).

4.4.2 Changes in the power of subcortical rhythms with age and AD-like pathology

To investigate the effect of age and disease progression on network oscillations, we determined the relative power of a number of physiologically important frequency bands. The majority of research to date has focused on correlating changes in network rhythms with the severity of cognitive impairment and disease progression in human AD patients (Brenner et al. 1988; Coben et al. 1983, 1985; Giaquinto and Nolfe, 1986; Rae-Grant et al. 1987; Soininen et al. 1982; Kowalski et al., 2001), and have reported general increases in the power of delta and theta rhythms, and a decrease within the alpha and beta frequency bands. Following FFT analysis, we extracted the relative power of the oscillation within delta, theta, alpha and beta frequency bands, and assessed the influence of age and AD-like pathology. This work represents one of the first studies that have assessed how hippocampal network oscillations are affected by age and disease, with the majority of research focusing on cortical EEG activity, and represents the only study to assess activity in the CA1 and subiculum (Witton et al., 2014; Scott et al., 2012).

We compared all rhythms (delta, theta, alpha, and beta) versus age and genotype by means of a factorial MANOVA. This allows for the determination of overall age and/or genotypic differences across all bands within each hippocampal region. Factorial MANOVA within the CA1 detected an overall geotypic (F(4, 28)=4.113,

2Factorial MANOVAs are unable to detect pair-wise genotypic difference by post-hoc tests due to there being fewer than three components (AD and control). Therefore, pair-wise bonferroni analyses were conducted to detect genotypic differences.
Figure 4.1. Illustration of experimental preparation. (A) Electrode configuration and placement within the HF. 32-channel multi-electrode recording arrays are inserted to span the dorsal-ventral axis of CA1 (left) and subiculum (right). (B) Examples of filtered-state recordings used in analysis. Top shows an example of a delta-filtered signal (0.2-2Hz) and bottom image shows an example theta-filtered signal (2.5-4.5Hz) (C) Example of spectrogram analysis over the entire recording (x-axis represents time from beginning to the end of the recording) (top), with state separation (bottom). Note how the spectrum fluctuates throughout the recording, oscillating between periods of delta (red) and theta (green) activity. White sections represent ‘undetermined’ periods within the LFP signal. LFP traces (D) illustrate delta- (top) and theta-dominant (bottom) periods as determined through spectral analysis. Time periods in which the delta- and theta-dominant periods were taken from to produce the images shown in D are represented by the black and blue boxes in C, respectively.
4.4. Results

p=0.01) and age-related change in the power across all rhythms (F(8,56)=5.138, p<0.0001). In addition, a factorial MANOVA also detected an overall genotypic (F(4,28)=5.116, p=0.0032) and age-related (F(8,56)=8.841, p<0.0001) change in power within the subiculum.

Following assessment by means of a MANOVA, two-way between subjects differences were detected. Assessment of delta power (two-way ANOVA; age and genotype as factors) revealed significant genotypic differences within CA1 (F(1,30)=13.12, p=0.0011) and subiculum (F(1,31)=15.75, p=0.0004), with a general increase in delta power observed in control mice at 3- and 6-months within CA1, and 6 months in subiculum. Our data also reveals an age-related change in delta power as a function of age in subiculum (F(2,31)=19.25, p<0.0001), with an age-related increase observed in control mice between 3- and 6 months, followed by a decrease between 6- and 9 months (Figure 4.2A).

Interestingly, two-way (age and genotype) ANOVA shows that there was no genotype-related difference for theta power in either region of the HF. However, there was an overall reduction in theta power as a function of age in both CA1 (F(2,31)=11, p=0.0002) and subiculum (F(2,31)=7.049, p=0.003) and a non-significant trend for elevated theta power in 3xTgAD mice when compared to controls (Figure 4.2B).

Two-way (age and genotype) ANOVA shows an age-related change in alpha rhythm power in both CA1 (F(2,31)=12.38, p=0.0001) and subiculum (F(2,31)=15.36, p<0.0001), with evidence of genotype related changes within the subiculum (F(1,31)=7.68, p=0.0118). Post-hoc tests reveal an age-related increase in power between 3-6 month mice in both genotypes, and a concurrent reduction between 6-9 months in control mice within CA1. We also detect an age-related increase in power within subiculum in both genotypes between 3-6 months, followed by a decrease between 6 and 9 months in 3xTgAD mice. Specific genotypic differences were detected at 6 months in the subiculum; with 3xTgAD mice displaying significantly elevated alpha power. Therefore, the 3xTgAD mice are likely to be showing signs of advanced ageing within the neuronal network of the HF (Figure 4.2C).

In agreement with the changes found in alpha power, two-way (age and genotype) ANOVA reveals an general age-related change in beta rhythm power in both CA1 (F(2,31)=19.99, p<0.0001) and subiculum (F(2,31)=16.81, p<0.0001). In addition, we detect an overall genotypic difference within subiculum (F(1,31)=5.508, p=0101). Post-hoc analysis reveals an age-related increase within CA1 between 3-6 months in both genotypes, followed by a decrease in power between 6-9 months in control mice, but a continued increase in 3xTgAD mice between 3-9 months. An age-related increase in beta power was also observed within subiculum, with an increase between 3-6 months present in both genotypes, followed by continued increase between 6-9
months in 3xTgAD mice. Genotype differences were observed at 9 months within CA1, and 6 months within subiculum, with a general increase in power in 3xTgAD mice. Again, this is representative of an advanced ageing like phenotype of the 3xTgAD mouse (Figure 4.2D).

4.4.3 Differences in the cross-covariance of theta rhythms

In order to determine the relationship between CA1 and subicular rhythms, we tested for cross-covariance between the two regions for both delta and theta filtered signals. Cross-covariance analyses was computed on delta- and theta-filtered signals between CA1 and subiculum for each animal. Age and genotype averages were computed and plotted. All values were normalised in order to directly compare the strength of correlation between the two signals, and averaged between animals. There was a linear relationship between these regions for both rhythms across age and genotype, i.e. both signals varied in correlation with one another (e.g. as the CA1 signal became more positive, so did the corresponding subicular signal) (Figure 4.3). Note that cross-covariance analysis clearly demonstrates linear relationships between two signals, but is unable to determine causality or discount for similar variations produced by a common input.

4.4.4 Deficits in the information carrying capacity of delta state dominant signals in 3-month 3xTgAD mice

In order to determine the direction and amount of information transferred between the CA1 and subiculum we implemented TE analysis. This allowed for us to quantify, for the first time, the amount of information transferred during different oscillatory states. As such, we build on other research that has predominantly focused on determining changes in network connectivity through inter-regional coherence.

Using the delta-dominant periods of the signal, we calculated TE values between CA1 and subiculum in each direction. These TE calculations discount for any potential influence of a common input.

At 3 months, we detected a large and significant reduction in the information carrying capacity of the delta-dominant signal. These differences were apparent in both directions; i.e. CA1→subiculum, and subiculum→CA1. Two-way (genotype and delay) ANOVA revealed an overall genotypic difference for information transfer between CA1 to subiculum in the raw EEG component of the signal (F(1,13)=15.436, p=0.0365). We also show that predictability decreased as a function of delay in both EEG and phase components of the signal (EEG: F(200,2600)=12.82, p<0.0001, phase:
Figure 4.2. Age and genotype related changes in network oscillations. (A) Power of delta rhythms (0.2-2.5Hz) as a function of age and genotype. (B) Power of theta rhythms (2.5-4.5Hz) as a function of age and genotype for both CA1 (left) and subiculum (right). (C) Power of alpha rhythms (9-16Hz) as a function of age and genotype for both CA1 (left) and subiculum (right). (D) Power of beta rhythms (16-30Hz) as a function of age and genotype for both CA1 (left) and subiculum (right). Inset shows an example FFT indicating the regions which were sampled for power analysis. Note that the faster frequencies (alpha and beta) have a much lower amplitude and are therefore difficult to visualise in FFT analysis. All data were analysed using a two-way (age and genotype) ANOVA followed by application of a Bonferroni post-hoc test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Errors are shown as ±SEM.
Figure 4.3. Cross-covariance between CA1 and subiculum for delta and theta rhythms. Normalised cross-covariance of CA1-subicular delta (0.2-2Hz) (left) and theta (2.5-4.5Hz) (right) rhythms in both control and 3xTgAD mice aged: (A) 3 months; (B) 6 months; and (C) 9 months.
4.4. Results

F(200,2600)=15.94, p<0.0001). In addition, two-way (genotype and delay) ANOVA showed that there was an overall reduction in information in the 3xTgAD mouse compared to control in both EEG and phase components of the LFP from subiculum to CA1 (EEG: \(F(1,13)=5.854, p=0.0309\), phase: \(F(1,13)=8.225, p=0.0132\)). The overall reduction in the predictability of past values of the target signal reduced as a function of delay when assessing the flow of information from subiculum to CA1 in both EEG (\(F(200,2600)=13.22, p<0.0001\)) and phase (\(F(200,2600)=15.63, p<0.0001\)) components of the LFP (Figure 4.4A). Our analysis was unable to detect any genotypic differences, in either direction, in amplitude measurements of the LFP.

As the detection of genotype differences is likely to be influenced by our arbitrary choice for the delay period, we also compared genotypes based on their maximal information values and mean information rates over the 1 second delay range. Two-way (genotype and direction) ANOVAs indicated that for peak information, there was an overall genotype difference in both EEG (\(F(1,26)=32.69, p<0.0001\)) and phase (\(F(1,26)=16.92, p=0.0003\)) components, with pair-wise differences evident in both directions. There were similar patterns for mean information, with more information in control mice for both EEG (\(F(1,26)=11.28, p=0.0024\)) and phase (\(F(1,26)=16.61, p=0.0004\)) components of the LFP signal. Here we detected pair-wise differences in both directions of information flow when assessing the phase component of the LFP and between subiculum and CA1 for the general EEG component.

We observed no genotypic differences in the information carrying capacity of the signal during delta-dominant periods in 9 month mice. However, genotypic differences are still apparent when assessing for overall genotypic and age-related differences in the peak and mean flow of information between these ages. When evaluating the flow between CA1 and subiculum we detect an overall genotypic difference in the mean EEG (\(F(1,22)=8.53, p=0.0079\)) and phase (\(F(1,22)=5.013, p=0.0356\)) components, and in the peak phase (\(F(1,22)=5.411, p=0.0296\)) component of the LFP signal. In addition, genotypic differences remain when assessing the flow of information between subiculum and CA1 in the mean EEG (\(F(1,22)=5.3, p=0.0312\)) and phase (\(F(1,22)=5.946, p=0.0233\)) components, and in the peak EEG (\(F(1,22)=11.2, p=0.0029\)) component of the LFP signal. Whilst overall genotypic differences were observed, pairwise differences were only detected between genotypes at 3 months of age (data not shown).
4.4. Results

Figure 4.4. Deficits in the flow of information during delta-dominant, non-filtered periods in the 3-month 3xTgAD mouse. (A) The flow of information from CA1 to subiculum (left) and subiculum to CA1 (right) for raw EEG (top) and phase (bottom) over the 1 second delay range. (B) Mean information (bits/s) flow for raw EEG (left) and phase (right) components of the LFP. (C) Peak information from the raw EEG (left) and phase (right) component of the LFP. Delay-dependant data (A) were analysed by two-way (genotype and delay) repeated measures ANOVA followed by application of LSD analysis to reveal pair-wise differences. Mean and Peak information data were analysed by two-way (genotype and direction) ANOVA followed by the application of post-hoc Bonferroni to detect pair-wise differences (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). For panels B and C: CA1=CA1→Subiculum; Subiculum=Subiculum→CA1. Errors are shown as ±SEM.
4.4.5 Deficits in 3-month 3xTgAD mice during delta dominant periods are predominately due to changes in the information carrying capacity of SWRs

During analysis of all components (raw EEG, phase and amplitude) of the LFP signal during delta/theta-dominant and filtered signals, the most prominent deficits for information transfer were apparent during delta-dominant periods. Note that amplitude and phase-based measurement determine the information carrying capacity of the signal based on the individual components of the LFP signal, whilst raw EEG-based does not determine this capacity based on a single feature of the waveform. We did not find any genotype difference for information capacity in delta-dominant activity when filtered for the large slow component (0.2-2Hz), supporting the conclusion that the slow-wave component of the delta signal does not contribute to the breakdown in information found during delta-dominant periods. In addition, the differential contribution of theta rhythms can be discounted as information capacity is unaffected during theta-dominant or theta filtered signals at 3 months (data not shown). As a result, we investigated the contribution of the higher frequency SWR components that are present during delta periods. As SWRs are known to be critical for the formation of long-term memories during non-REM delta sleep, and therefore warrant the investigation of this component of the LFP signal. To demonstrate the importance of investigating other frequency components during the delta signal, two-way (genotype and delay) ANOVAs reveal that there are no genotype related changes in CA1→subiculum information capacity in delta-filtered signals in any component of the signal in 3 month mice (raw EEG, phase and amplitude) (Figure 4.5A: Mean (left) F(1,39)=1.233, p=0.2736, Peak (right) F(1,39)=1.212, p=0.2776). Therefore, it is likely that another co-existing frequency component must contribute to this change in information, other than the slow-wave component. In support of this, we show that SWRs co-occur with the on-going slow-wave rhythm and therefore confirm that this frequency component is present during delta-dominant periods of the signal (Figure 4.5B). In order to investigate the contribution of the SWRs in the CA1/subiculum pathway we applied a band-pass filter (120-180Hz) to obtain an LFP signal that only contained components within the SWR frequency band. We then merged the delta- and SWR-filtered signals in order to confirm that the slow-wave components co-occur with SWRs.

Two-way (genotype and direction) ANOVAs revealed a strong and significant difference in the information capacity of the CA1-subicular network during SWR activity for both mean (F(1,26)=438.8, p<0.0001) and peak (F(1,26)=122.4, p<0.0001) measurements of TE (Figure 4.5C). Pair-wise differences were detected in both directions of information flow and in both measurements. This provides strong
support for a breakdown in functional connectivity between these regions during delta rhythms, specifically during fast frequency SWR component of the signal.

4.4.6 Deficits in the information capacity of SWR oscillations are not due to a lack of SWR oscillation in 3xTgAD mice

Both control and 3xTgAD mice expressed SWR oscillations during periods of delta activity under urethane anaesthesia (Figure 4.6). Due to the large and significant differences in the information carrying capacity observed, particularly during SWR oscillations, we assessed the power and patterns of SWRs in each group. Two-way (genotype and region) ANOVAs show that there were no differences in the power of the frequency band within the SWR range (120-180Hz) and, therefore, deficits in SWR-related information in 3xTgAD mice are unlikely to be due to differences in LFP power (Figure 4.7). To evaluate the pattern of SWR activity, we performed perievent analysis, triggered to delta troughs, and autocorrelation analysis to evaluate SWR rhythmicity. Unfortunately, the results to the perievent analysis were noisy; most likely due a lack of SWR entrainment to a particular phase of the delta rhythm. However, autocorrelation analysis demonstrated that both 3xTgAD and matched control mice showed similar rhythmicity and patterns of SWR activity in CA1 and subiculum. Therefore, we can discount for a lack of SWR activity as a confounding factor in 3xTgAD mice in relation to their low information transfer during SWR periods.

4.4.7 Deficits become apparent in the filtered theta signals in the 3xTgAD mouse by 6 months

In order to investigate the effect of further age and AD-like pathology progression on the flow of information through the CA1-subicular pathway, we assessed the information capacity of the delta and theta rhythms in 6-month 3xTgAD and aged-matched control mouse. We report that by 6 months there is a change in the network connectivity from that found at 3 months, with a deficit becoming apparent in theta-filtered signals of the LFP.

At 6 months, we detected a large and significant increase in the information carrying capacity of the theta-filtered signal in 3xTgAD mice, with differences being apparent in both directions (Figure 4.8). Two-way (genotype and delay) ANOVAs showed that over a one second delay range there was an overall genotype difference between CA1→subiculum in EEG (F(1,8)=5.594, p=0.0456) and phase (F(1,8)=7.268, p=0.0272) components of the LFP signal. In both EEG and phase measurements
4.4. Results

Figure 4.5. SWRs are a key component of the delta-dominant, non-filtered signal involved in the transfer of information in the CA1-subicular pathway in 3 month mice. (A) Mean (left) and peak (right) information in all components (raw EEG, phase and amplitude) of the delta filtered (0.2-2Hz) signal for CA1-subicular information. Note that there are no detectable genotypic differences for any measure. (B) Overlay of a delta- and SWR-filtered components to show the co-occurrence of both low and high-frequency components during non-filtered delta-dominant periods. (C) Mean (left) and Peak (right) information from SWR-filtered signals. Note the large and significant genotypic differences in both directions and for both measurements, with 3xTgAD showing consistently lower information levels. Mean and Peak information data were analysed by two-way (genotype and direction) ANOVA followed by the application of post-hoc Bonferroni to detect pair-wise differences (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). CA1= CA1→Subiculum, Subiculum= Subiculum→CA1. Errors are shown as ±SEM.
Figure 4.6. Autocorrelation analysis of SWRs in 3 month mice. Autocorrelation analysis illustrates that 3 month control and 3xTgAD mice exhibit rhythmic SWR activity in CA1 and subiculum.

Figure 4.7. Regional power of SWR oscillations in 3 month mice. The power within the SWR frequency band (120-180Hz) is similar between genotypes and regions.
there was a general decrease in the predictability of the past values of the signal in relation to the other as the delay (tau) increased (EEG: $F(200,1600)=11.26$, $p<0.0001$, phase: $F(200,1600)=5.7$, $p<0.0001$). In addition, there was an overall increase in information in the 3xTgAD mouse in the phase component of the LFP between subiculum→CA1 (phase: $F(1,8)=9.294$, $p=0.0159$). The overall predictability of the past responses of the target signal is also seen to reduce as a function of delay between subiculum→CA1 in both EEG ($F(200,1600)=9.267$, $p<0.0001$) and phase ($F(200,1600)=3.837$, $p<0.0001$) components of the LFP (Figure 4.8A).

As the detection of overall genotype differences are likely to be influenced by the arbitrary choice of delay range, we also compared genotypes based on their peak information values and mean (Figure 4.8C and 4.8B, respectively) information rates over the entire 1 second period. Two-way (genotype and direction) ANOVAs revealed an overall genotype difference for peak information in both EEG ($F(1,16)=19.08$, $p<0.0005$) and phase ($F(1,16)=23.52$, $p=0.0002$) components, with pair-wise differences evident in both directions, as detected through the application of Bonferroni post-hoc test for differences (data not shown). Two-way (genotype and direction) ANOVAs also showed that there were similar patterns based on mean information, with increased information in control mice based on both EEG ($F(1,16)=10.76$, $p=0.0047$) and phase ($F(1,16)=15.15$, $p=0.0013$) components. Again, we detect pair-wise differences in both directions, as detected by post-hoc Bonferroni test for differences.

In addition, we computed the information carrying capacity of the network based on theta-filtered signals between CA1→subiculum and subiculum→CA1, in 3- and 9-month 3xTgAD and control mice (data not shown). Based on the information values obtained from EEG and phase components of the LFP signal, we assessed how information flow changes based on mean and peak TE values as a function of age and genotype. Two-way (genotype and age) ANOVAs showed that there was an overall decrease in the information carrying capacity as a function of age but no evidence of genotype differences between CA1 and subiculum for mean and peak information values (Mean: EEG $F(2,28)=9.421$, $p=0.0007$ and phase $F(1,28)=10.28$, $p=0.0004$, Peak: EEG $F(2,28)=10.04$, $p=0.0005$, phase $F(2,28)=11.71$, $p=0.0002$) and between subiculum and CA1 for both mean and peak values (Mean: EEG $F(1,28)=10.02$, $p=0.0005$, phase $F(1,28)=11.85$, $p=0.0002$, Peak: EEG $F(1,28)=10.34$ $p=0.0004$, phase $F(1,28)=10.39$, $p=0.0004$).
Figure 4.8. Increased flow of information during theta filtered periods in the 6-month 3xTgAD mouse. (A) Flow of information over 1s delay range between CA1 and subiculum based on the raw EEG (top) and phase (bottom) components. There is general reduction in the predictability of the target signal with increasing delay. (B) Mean information (bits/s) flow between CA1 and subiculum based on raw EEG (left) and phase (right) components of the LFP. (C) Peak information values in control and 3xTgD mice based on analysis from the raw EEG (left) and phase (right) component of the LFP. Time dependant data (A) were analysed by two-way (genotype and delay) repeated measures ANOVA followed by application of LSD analysis to reveal pair-wise differences. Mean and Peak information data were analysed by two-way (genotype and direction) ANOVA followed by the application of post-hoc Bonferroni tests to detect pair-wise differences (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). CA1= CA1→Subiculum, Subiculum= Subiculum→CA1. Errors are shown as ±SEM.
4.4.8 3xTgAD mice show deficits in the slow-wave component of delta rhythms at 9 months

At 9 months we detected subtle deficits in the slow-wave component of the delta signal in 3xTgAD mice. Unlike at younger ages (Figures 4.4-4.6) we could only detect genotypic differences in the raw EEG component of the signal, that is, the phase components of the signal were similar (data not shown). Two-way (genotype and delay) ANOVAs revealed that there were genotypic differences in the delta-filtered (0.2-2Hz) raw EEG signal over the delay range (0-1000ms) in the CA1 to subiculum direction (F(1,9)=5.342, p=0.0461) and the subiculum to CA1 direction (F(1,9)=6.684, p=0.0294) (Figure 4.9A). In agreement with analyses at younger ages, there was an overall reduction in the predictability of the past values of the target signal with increasing delay (CA1-subiculum (F(200,1800)=81.29, p<0.0001, subiculum-CA1 (F(200,1800)=74.43, p<0.0001).

To test the dynamics of information flow by other measures, we assessed the differences in information as determined by mean and peak information values through the application of two-way (genotype and direction) ANOVAs, followed by post-hoc Bonferroni test for differences (Figure 4.9B). Whilst there were genotype differences in the mean information values (F(1,18)=11.88, p=0.0029) we were unable to detect any pair-wise differences. However, peak information values appeared to be more informative as there was both an overall effect of genotype (F(1,18)=12.91, p=0.0021) and pair-wise differences in both directions of information flow, with greater amounts of information present in control mice. Whilst we detected genotypic differences in the theta-filtered signal at 6 months (Figure 4.8), by 9 months these are similar, suggesting an age-related and generalised decrease in theta-related information (Figure 4.9C). To demonstrate the lack of any detectable genotypic-related change in transfer entropy during theta-dominant periods at 3 and 9 months, raw transfer entropy graphs are shown in Figure 4.10.

4.5 Discussion

This work represents the first of its kind to investigate the causal relationships of the LFP between CA1 and subiculum, and investigate how this alters as a function of age, and AD-like pathology progression. Our results indicate that there are early deficits in the information carrying capacity of the hippocampus (HC), as revealed by analysis of causal information transfer between CA1 and subiculum during spontaneous EEG activity in anaesthetised 3xTgAD mice. We show that we are able to detect a large and significant deficit in the bidirectional flow of information as early as 3
Figure 4.9. Deficits in the flow of information during delta filtered periods in the 9-month 3xTgAD mouse. (A) Flow of information between CA1 and subiculum based on the raw EEG over the 1s delay range. There was a general reduction in the predictability of the target signal at longer delays. (B) Mean (left) and Peak (right) information (bits/s) between CA1 and subiculum. (C) Information capacity of theta-filtered signals in all components (raw EEG, phase and amplitude) at 9 months. Note that there are no genotypic differences in either component as measured by either mean (left) or peak (right) information values. Time dependent data (A) were analysed by two-way (genotype and delay) repeated measures ANOVA followed by application of LSD analysis to reveal pair-wise differences. Mean and Peak information data were analysed by two-way (genotype and direction) ANOVA followed by the application of post-hoc Bonferroni to detect pair-wise differences (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). CA1= CA1→Subiculum, Subiculum= Subiculum→CA1. Errors are shown as ±SEM.
Figure 4.10. Transfer entropy is similar between genotypes during periods of theta in 3 and 9 month mice. Top panels show the flow and amount of information between CA1 and subiculum in 3 month control and 3xTgAD mice during the raw (left panels), phase (middle panels) and energy (right panels) components of the EEG signal. Similarly, the bottom panels show the same information in 9 month control and 3xTgAD mice. Errors are shown as ±SEM.
months, and that this change is initially specific to the delta periods of the network oscillation. We further report that these differences are not due to abrupt changes in the slow-wave rhythm, but are due to a breakdown in communication during the faster SWR frequencies. We also demonstrate that this is not simply due to the loss of, or changes in rhythmicity of the SWR band, and therefore is likely to represent a change in the network dynamics/processing during SWR periods. This study therefore provides the first evidence of a direct measureable change in the network dynamics that correlates with the onset of cognitive decline in the 3xTgAD mouse. We further show that the network dynamics and information processing capacity alters as a result of age and AD-like pathology, and therefore may provide a useful tool in the detection and staging of AD.

The processing and storage of memories requires highly co-ordinated and synchronous activity of large populations of neurons. One possible mechanism to orchestrate this function is through the highly co-ordinated activity of neurons, generating large neuronal oscillations such as, theta, delta and gamma (Buzsáki, 2002). To date, the majority of research focusing on the assessment of neuronal function and detecting age- and disease-related changes has done so through the analysis of power and coherence of these rhythms (Brenner et al., 1988; Coben et al., 1983, 1985; Giaquinto and Nolfe, 1986; Rae-Grant et al., 1987; Soininen et al., 1982; Kowalski et al., 2001).

Those that have conducted studies using mouse models of AD have indicated a general decrease in the power of theta, delta and gamma rhythms (Wang et al., 2002; Rubio et al., 2012; Scott et al., 2012). However, this assessment has not been conducted on the 3xTgAD mouse. In agreement with other mouse studies, we detected an overall age- and genotype-related decrease in the power of hippocampal delta and an age-related decrease in theta (Wang et al., 2002; Rubio et al., 2012; Scott et al., 2012), and an age- and genotype-related increase in the power of alpha and beta rhythms (Wang et al., 2002) (Figure 4.2). However, this and other mouse studies seem to contradict human studies, where there is a general increase in the power of cortical delta and theta rhythms, and a decrease in alpha and beta (Brenner et al., 1988; Coben et al., 1983, 1985; Giaquinto and Nolfe, 1986; Rae-Grant et al., 1987; Soininen et al., 1982; Kowalski et al., 2001). Therefore it is important to consider the experimental differences that might explain this apparent disparity. Notably, as most human studies record EEG at the scalp, these will be dominated by cortical EEG changes which are likely to correlate more strongly with cortical rather than sub-cortical dementia (Verma et al., 1987; Jeong et al., 2001). In addition, human AD studies have shown degradation in the cholinergic system thought to account for changes in network rhythms (Schliebs and Arendt, 2006), yet this cholinergic loss has not been reported in AD mice (Liu et al., 2002; Wong et al., 1999). Instead,
reductions in GABAergic septo-hippocampal connectivity have been suggested to account for the changes in rhythmicity in AD mouse models (Rubio et al., 2012).

Whilst EEG power and correlation analyses provide us with some understanding of the network changes associated with neuropsychiatric states such as AD, these approaches cannot determine inter-regional causality or discount for the effect of common input. The present study addresses these limitations for the first time in an AD model using a computational approach to determine the direction and magnitude of information carrying capacity within the CA1-subiculum network during spontaneous EEG recordings in vivo. In this, we build on Shannon entropy and Granger causality by the application of TE analysis during different oscillatory states in CA1 and subiculum. In all of our analyses we observed an expected strong causal influence of CA1 activity on that recorded in subiculum. This is supported by the well-documented major projection from CA1 to subiculum (Witter and Amaral, 1991; Amaral and Witter, 1989; Commins et al., 1998). However, we also observed a similar influence of subiculum over CA1 activity. Whilst this may seem surprising, there is increasing anatomical and functional evidence for strong excitatory and inhibitory subicular outputs that terminate directly in CA1 (Jackson et al., 2014; Sun et al., 2014). Thus, recent work by Jackson and colleagues has indicated that subicular theta rhythms lead those in CA3, with GABAergic back-projections from subiculum involved in CA3 theta modulation (Jackson et al., 2014). Through the use of Granger causality these authors also showed that during theta rhythms net information flows from subiculum to CA1 (Jackson et al., 2014). However, since Granger causality cannot discount for the influence of a common input (such as the connection from entorhinal cortex layer III to subiculum and CA1), our TE results provide stronger support for subiculum as a hippocampal input region, as TE discounts any effect of co-modulation from common inputs.

Our results reveal deficits specific to delta activity, and in particular to the delta-modulated higher frequency SWRs at 3 months in the 3xTgAD mice (Figure 4.4 and 4.5). This delta and SWR specific deficit is likely to result in severe consequences for the consolidation of hippocampal-dependent memories. Studies have indicated that the HC acts as a short-term buffer for memories, after which memories are transferred to the neocortex for long-term consolidation. Theta rhythms are involved in the acquisition and retrieval of memories, and slow-wave/delta rhythms are involved in the consolidation of these memories to the neocortex (Hasselmo et al., 2002; Born et al., 2006; Stickgold and Walker, 2005; Diekelmann and Born, 2010; Molle and Born, 2011; Walker and Stickgold, 2004). Specifically, research has shown that SWRs are a key component of the signal during non-REM (delta) sleep and
are vital for this consolidation process (Sirota et al., 2003; Girardeau et al., 2009; Ego-Stengel and Wilson, 2010; Buzsáki, 1989; Logothetis et al., 2012). In further support of the role of SWRs in the consolidation process, CA1 place cells are found to ‘replay’ their activity during periods of slow-wave sleep, and that their discharge is associated with sharp-wave activity (Pavlides and Winson, 1989; Wilson and McNaughton, 1994). This sequential ‘replay’ of CA1 cells is found to fire at a higher rate during SWR activity, and that this ripple activity is an ideal candidate for long-term potentiation (Buzsáki, 1989; Skaggs et al., 1996; Lee and Wilson, 2002; Steriade et al., 1993). Furthermore, disruption of SWR activity in the HC during the consolidation phases of various hippocampal-dependent tasks is found to result in robust consolidation deficits (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010).

In support of these early network deficits, recent work conducted on the TgCRND8 (APP) mouse observed deficits in the theta-gamma coupling in the subiculum as early as 1 month (Goutagny et al., 2013). Additionally, the importance of sleep, and the effect of disturbances on memory performance has been reported in AD patients. AD patients typically report disturbances in their sleep pattern, with alterations in long-term memory retention being one of the first cognitive changes during the early stages of the disease. As such, we propose that this early deficit in the performance of the CA1-subiculum pathway is likely to facilitate the cognitive deficits observed at this stage in the 3xTgAD mouse, such as the those found by Billings et al. (2005), where 3xTgAD mice acquire but fail to consolidate memories during spatial tasks. This deficit, along with the synaptic deficits observed in the CA1, DG and subicular subfields (Davis et al., 2014), occurs prior to the observation of overt AD-like pathology, and therefore implicates the roles of oligomeric Aβ in the development of neuronal deficits (Palop and Mucke, 2010b,a; Palop et al., 2007; Oddo et al., 2003; Billings et al., 2005; Mastrangelo and Bowers, 2008).

We find that deficits in the information carrying capacity of the CA1-subiculum pathway are complex, and alter with age and disease progression. We show that by 6 months, 3xTgAD mice show evidence of network changes in the theta signal. Specifically, we observe a net increase in information flow between CA1 and subiculum, significantly above that observed in control mice (Figure 4.8). Whilst it may be initially thought that an increase in information is beneficial, this may not necessarily be the case. Neuronal activity of the HC is under tight control of inhibitory and excitatory neurons, which is essential to controlling the fine-tuning of the rhythmic activity observed in this structure. Therefore, it is conceivable that a small change in the system will alter the dynamics of the HC. We propose that the presumed loss of inhibitory control observed in the EC-DG-CA1 pathway in 3xTgAD mice aged 4-6 months results in hippocampal hyperexcitability, likely ‘overloading’ the system (Davis et al., 2014). This deficit in the flow of information, coupled with
hippocampal hyper- and hypoexcitability at 6 months, is likely to contribute to the hippocampal-dependent episodic-like memory deficits in the 3xTgAD mouse (Davis et al., 2013b,a).

By 9 months of age we show that 3xTgAD mice display a subtle, but significant, deficit in the slow-wave component of the delta signal, and that information conveyed by the theta rhythm becomes similar to that in controls (Figure 4.9). At this age there is no difference in information flow based on the phase of EEGs, therefore, this stage is likely to represent an advanced ageing and disease phenotype. This is in agreement with other reports that at this age control mice are similar to 3xTgAD mice, for both synaptic excitability and cognitive measures (see previous chapter) (Davis et al., 2013b,a).

We also conclude that changes in neuronal communication are unlikely to be contributed by the effect of urethane anaesthesia. In agreement with other studies, we find that urethane-induced anaesthesia induces long-term and stable levels of unconsciousness that closely mimic that of natural sleep (Clement et al., 2008). We find evidence of multiple transitions between theta and delta activity, where information flow is observed between CA1 and subiculum during both oscillations. Therefore, for the first time, we were able to determine causality of a number of spontaneous EEG rhythms and quantify the amount of information transferred through the CA1-subiculum fields of the HF. There is an early and robust deficit in the information carrying capacity in the EEG of 3xTgAD mice as early as 3 months of age. This deficit in neuronal communication and the specific alterations in the SWR/delta signal correlate with the onset of electrophysiological and cognitive deficits in other studies and with the development of intracellular Aβ (Oddo et al., 2003; Billings et al., 2005; Davis et al., 2013b,a, 2014; Mastrangelo and Bowers, 2008).
Chapter 5

Discussion

5.1 Overview

AD is a neurodegenerative condition of the central nervous system, and is the most common form of all dementing illnesses. This progressive degeneration results in the gradual loss of cognition, in particular a decline in declarative memory formation and retrieval (McKhann et al., 1984, 2011). AD patients will initially report symptoms of subtle disturbances in short-term declarative memory, which is clinically diagnosed as MCI. In the majority of cases MCI progresses to AD with increased degeneration through development of AD pathology and increased severity of cognitive and emotional impairments. The HF is critical for the formation and retrieval of declarative memories and spatial navigation (Scoville and Milner, 1957; Milner, 1959; Penfield and Milner, 1958; Eichenbaum, 2000), and is one of the first regions affected by AD pathology and neuronal degeneration (Braak and Braak, 1991, 1997). However, little is understood as to how the onset and further progression of AD-related pathology affects neuronal function within the HF. Therefore, it is vital to further develop our understanding of the mechanisms of the disease and how this impacts effective network connectivity and function within the HF.

In order to investigate the effect of AD on neuronal connectivity and network communication directly, we must make use of established animal models of disease. Currently, the majority of AD models are developed in mice, with only one known model in rats (Cohen et al., 2013). This allows us to probe neuronal network function within a system that expresses predictable states of disease progression. As such, the stereotypic pathology development in mouse models provides a degree of experimental control that is extremely difficult to obtain in human studies, as these typically investigate patient cohorts where individuals are at differing stages of AD. Therefore, these mouse models allow us to investigate the effects of AD-like pathology
5.1. Overview
during the early/prodromal stages of the disease. In this study I conducted research
using the 3xTgAD mouse, a well-established mouse model that develops cognitive
deficits and AD-related pathology in a similar temporal and spatial manner to that
in human patients (Oddo et al., 2003; Billings et al., 2005; Davis et al., 2013b,a;
Mastrangelo and Bowers, 2008). In addition, this reflects one of the few models to
develop tau pathology (albeit from a genetic mutation related to fronto-temporal
dementia) and therefore represents a model that expresses the full AD pathology
profile (Mastrangelo and Bowers, 2008; Oddo et al., 2003).

In both the 3xTgAD mouse and human form of AD, pathology develops in a
well-defined temporal and spatial pattern. Beta-amyloid plaque pathology is first
detected within the cortex which later progresses to the HF, whereas tau pathology
first develops within the HF and then progresses to the cortical regions (Braak and
Braak, 1991, 1997; Mastrangelo and Bowers, 2008). However, both intracellular and
extracellular Aβ, and tau pathology develop first within the EC and CA1/subiculum
border of the HF. The CA1 and subiculum represent the final regions of the HF
prior to the output of hippocampal activity to the neocortex, and are two of the
first regions to become heavily affected by AD pathology (Mastrangelo and Bowers,
2008). However, with growing evidence challenging the traditional view that the
subiculum merely acts as a hippocampal output region, and that it may in fact also
serve to supply hippocampal input, it has received relatively little attention, with no
known research investigating the impact of AD pathology (Deadwyler and Hampson,
2004; Jackson et al., 2014). This study focused on assessing how neuronal function
is affected as a result of AD-like pathology and compared to the changes associated
with the normal ageing process.

Specifically, the aims of this study were to:

1. Determine the onset and development of synaptic deficits between CA1 and
   subiculum in 3xTgD mice through assessment of input-output relationships,
   short- and long-term plasticity.

2. Determine how spectral changes in network oscillations (EEG/LFP) change as
   a function of age and AD-like disease progression, and how this correlates with
   synaptic and network deficits.

3. Assess changes in the information carrying capacity of spontaneous network
   oscillations between CA1 and subiculum through the application of information
   theoretic analysis techniques.
5.2 Changes in synaptic connectivity in the CA1-subicular network

Currently, there has been only limited in vivo electrophysiological research for the 3xTgAD (Davis et al., 2014) and other mouse models (Gureviciene et al., 2004; Gengler et al., 2010). To date, the 3xTgAD in vitro preparation has shown a reduction in basal communication between CA3 and CA1, as demonstrated by a rightward and downward shift in the I/O curve (Oddo et al., 2003). Additionally, this work indicated impaired LTP induction and maintenance, correlating with the development of intracellular Aβ pathology and spatial memory deficits (Oddo et al., 2003; Billings et al., 2005). More recent work conducted an in vivo study to investigate hippocampal circuitry during early (4-6m) and late (17-18m) stages of disease pathology (Davis et al., 2014). Specifically, the integrity of the PP input to DG and CA1 was assessed following subicular stimulation and presumed EC re-entrance (Davis et al., 2014; Kloosterman et al., 2004, 2003). This work indicated no evidence of alterations in basal connectivity, which contrasts with other studies conducted in other models (Chapman et al., 1999; Fitzjohn et al., 2001; Jacobsen et al., 2006; Oddo et al., 2003).

In this study, I conducted an in vivo assessment of neuronal function in the comparatively under-investigated CA1-subiculum pathway. This study revealed that the basal communication between CA1 and subiculum is intact, and therefore any differences are not merely due to changes in the basic neuronal connectivity. I show an overall age-related decrease in basal connectivity, evident from 6 months, although we do not report any significant genotype differences. As such, alterations in basal synaptic connectivity are subtle, and only evident as a group difference. Our study confirms the view that the 3xTgAD mouse is free from overt functional synaptic abnormalities at birth (Oddo et al., 2003; Davis et al., 2014). In agreement with our work, changes in basal connectivity have also been reported in other in vitro studies, albeit in other models (Fitzjohn et al., 2001; Hsia et al., 1999; Oddo et al., 2003). However, this finding disagrees with other reports (Davis et al., 2014; Chapman et al., 1999), although these discrepancies are most likely due to differences in the experimental preparation and regions concerned. In further support, I show ‘normal’ spatial activation profiles for laminar recordings across the subiculum, illustrating CA1 inputs arriving in the cell layer, and possible feedback/lateral connectivity (Menendez de la Prida and Gal, 2004). Therefore, the spatial pattern of subicular connectivity remains functionally intact with both age and disease progression.

Through the assessment of PPF, I show a strong and significant reduction in short-term plasticity in subiculum as early as 3 months in the 3xTgAD mouse.
This PPF deficit is apparent predominately in the EPSP amplitude measurements, with only one pair-wise difference apparent in slope measurements. This early and robust decrease in short-term plasticity seems to decrease as a function of both age and AD-like pathology progression. In this study I show that 3xTgAD mice have significantly reduced PPF at 6 months, and by 9 months, both genotypes facilitate to similar levels with a significant drop as a function of age in control mice. In terms of PPF, I propose that 3xTgAD mice display an advanced ageing-like phenotype, with control mice eventually dropping to similar levels as those observed in the ‘young’ 3xTgAD mice. Again, this supports other studies that show a reduction in facilitation (Jacobsen et al., 2006; Gengler et al., 2010) and with one study supporting a loss of inhibitory control, and therefore hyperfacilitation, in the DG/CA1 regions (Davis et al., 2014). Researchers have proposed that the 3xTgAD mouse shows gross changes in calcium handling, most likely due to the effect of the PS1 transgene (Oddo et al., 2003; Smith et al., 2005; Lopez et al., 2008), and is therefore likely to directly impact neuronal communications. However, studies have shown that the addition of the APP transgene seems to negate these calcium-handling alterations. Here, I propose that changes in neuronal facilitation are unlikely to be contributed by changes in pre-synaptic calcium handling, or due to conductance changes reported by other studies (Desai et al., 2009). I demonstrate that I/O relationships are unaffected at 3 months, and are relatively unaffected during later stages of AD-like pathology. Additionally, I do not detect any short-term synaptic abnormalities (apart from in LTP) for EPSP slope measurements. All of the detectable abnormalities are for EPSP amplitude, suggesting that CA1 inputs to subiculum remain intact, and that subicular dendrites are activated by CA1 input similarly to those in control mice. As such, release probability appears normal in the CA1-subiculum pathway. However, it is possible that there is a general pre-synaptic failure, i.e. due to a reduction in maximal calcium influx, neurotransmitter concentrations, or alterations in the docking/release mechanisms.

Through the application of a repetitive LFS to CA1 that is known to evoke neuronal facilitation (and reverberation/re-entrance) in EC in rats (Kloosterman et al., 2003; Kloosterman et al., 2004), I further demonstrated significantly reduced neuronal facilitation in the 3xTgAD subiculum, evident as early as 3 months of age. This is in contrast to Davis and colleagues who demonstrated hyper-excitability in 3xTgAD mice, as shown by increased synaptic facilitation to LFS (and PPF) and an increased propensity to fire population spikes in DG/CA1 following stimulation of the PP (Davis et al., 2014). In the current study, to further support our theory of hypoexcitability, I am unable to report any evidence of population spikes, or increased neuronal reverberation.

Using the evoked responses to the repetitive LFS, I further investigate neuronal re-entrance through the application of CSD analysis. This allows for the visual
determination of the spatial and temporal flow of evoked synaptic currents within the subiculum upon CA1 stimulation. This current study illustrates the presence of a small but prominent CSD source within the deeper regions of the dendritic layer of subiculum, likely reflecting some reverberation through the hippocampal circuitry. However, in contrast to data reported within other systems, and hippocampal regions, we show that the subiculum is relatively refractory to hippocampal re-entrance. Here, I show that there is relatively weak reverberation through the hippocampal-subiculum circuitry, with a slightly decreased latency in 3xTgAD mice. Therefore, it is likely that the subiculum is under relatively high inhibitory control and as such resists neuronal reverberation. The results from this study, such as the reduced levels of PPF observed in amplitude measurements in 3xTgAD mice, indicate that there is an upregulation of the inhibitory tone within the subiculum of 3xTgAD mice. However, we were unable to find a direct measurable neuronal correlate to support this. To determine if the proposed change in inhibitory control is related to a change in the speed of the repolarising phase of the EPSP, we evaluated the upward slope values of the EPSP following PPF. In this analysis we were unable to detect any genotypic differences, and therefore can determine that the speed of inhibition in unlikely to be unaffected by AD-like pathology in this model.

Due to the differences observed between studies in terms of the age-of-onset and how neuronal function changes with AD-like pathology, results from this and other studies suggest that the hippocampal circuitry is not affected in a universal manner during AD, and is most likely regional and time-specific in relation to the underlying pathological state of the brain. Studies have shown that the development of pathology within the HF occurs in a well defined temporal and spatial manner, and therefore this is, at least to some extent, likely to account for some of the electrophysiological differences observed within different sub-regions as assessed at the same time-point since AD onset (Braak and Braak, 1991; Mastrangelo and Bowers, 2008).

In support of other studies, these results show evidence of an early and pronounced impairment in LTP induction, evident at both 3- and 6 months (Chapman et al., 1999; Fitzjohn et al., 2001; Gengler et al., 2010; Gureviciene et al., 2004; Jacobsen et al., 2006; Oddo et al., 2003; Wang et al., 2009). This further supports our work suggesting that there is a significant reduction in facilitation during early stages of AD. Such large-scale deficits becoming apparent during such early stages of disease progression is clearly of great importance for clinicians. This report demonstrates that changes in synaptic connectivity and synaptic facilitation are present prior to the development of overt A\(\beta\) and tau pathology, and prior to evidence of severe cognitive impairments (Mastrangelo and Bowers, 2008). However, they do correlate with the onset of subtle changes of selective hippocampal-dependant episodic-like memory tasks; although 3xTgAD mice perform at levels above chance at 3 months
of age they are significantly impaired compared to controls (Davis et al., 2013b,a). In addition, these early, yet significant impairments in both short- and long-term facilitation correlate well with the onset of deficits in memory consolidation (Billings et al., 2005).

In agreement with other studies, I propose that these neuronal changes correlate with the development of intracellular forms of Aβ (Oddo et al., 2003; Mastrangelo and Bowers, 2008; Billings et al., 2005). Indeed, studies have indicated that Aβ oligomers have a central role in neurotoxicity and resulting morphological changes in astrocytes and neurotransmitter alterations, resulting in the downregulation of synaptic activity (Lambert et al., 1998; Hsia et al., 1999; Hardy and Selkoe, 2002; Kamenetz et al., 2003; Jo et al., 2014). Additionally, application of Aβ oligomers results in a reduction of mini EPSPs, loss of AMPA receptors and a concurrent loss of dendritic spines (Shankar et al., 2007, 2008; Li et al., 2009). Therefore, Aβ oligomer induced changes typically result in a general reduction in glutamatergic neurotransmission that is likely to produce a deficit in LTP induction due to reduced AMPA and subsequent NMDA channel activation (Isaac et al., 1995). In addition, recent work has implicated the role of Aβ-induced astrocytic activation leading to upregulation in the underlying inhibitory tone (Jo et al., 2014). Whilst ‘normal’ astrocytes do not contain GABA, recent evidence has shown that Aβ can alter the phenotype of these cells, changing them to a ‘diseased’ state. This leads to the activation of these cells, leading to the production of GABA. This is then in turn released into the extracellular space, increasing the underlying inhibitory tone (Jo et al., 2014). I propose that as low-frequency synaptic transmission is maintained (3xTgAD input-output EPSP slope measurements are normal), that there is a general increase in the inhibitory tone within the subiculum during the early stages of AD. This likely results in the observed deficits in synaptic facilitation during stimulus pairs (PPF of EPSP amplitude) and stimulus trains at low-frequency (re-entrance) and high-frequency (LTP deficit). Whilst the results in this study are likely to be contributed to by a change in the inhibitory tone within the subiculum, it is unlikely to be due to a change in the relative strength of inhibitory feedback. Here, I show that the ascending phase of the evoked EPSP remains unchanged in relation to the slope values measured in control mice. Therefore, the relative speed of the repolarising phase of the EPSPs is similar between genotypes. These changes, although requiring further investigation, could be contributed to by an increase in the sensitivity of inhibitory neurons, leading to earlier inhibition of the EPSP, a general increase in the inhibitory tone, or other changes in the postsynaptic neuronal dynamics.

In summary, there is a complex and dynamic change in neuronal connectivity during the early stages of AD prior to the development of clinical pathology, but correlating with the development of intracellular forms of Aβ. The early hyper-
activity of the DG and CA1 regions during concurrent hypoactivity observed in the CA1/subicular pathway agrees with Aβ-induced changes proposed by Palop and Mucke (Palop and Mucke, 2010b,a). They propose that intracellular oligomers initially produces a period of hyperexcitability due to a decrease in inhibition, and a later period of hypoexcitability due to a decrease in glutamatergic transmission (Palop and Mucke, 2010b,a). However, it is important to consider that whilst these results show complex and pathology-dependent changes in neuronal dynamics within the HF, I do not detect a period of hyperexcitability in the CA1-subiculum pathway at 3 months. Instead, it is apparent that, by 3 months, AD-like pathology has advanced to the later stage of hypoexcitability in the Palop and Mucke model. In order to validate their proposition, we would need to conduct in vivo electrophysiological recordings in mice younger than 3 months. This would allow for the full profiling of the network changes to be investigated and correlated in relation to the changes observed within other regions of the HF.

5.3 Age and disease related changes in the spectral power of spontaneous network oscillations

EEG analyses are currently used as a diagnostic tool for a number of neurological conditions, including epilepsy and a variety of sleep disorders (Platt and Riedel, 2011). Due to its relatively low cost and ease of use, coupled with its non-invasive basis, it would be of great benefit to implement EEG spectral analysis as a potential biomarker for human AD. However, data arising from human and animal studies have provided mixed results (Jelic and Kowalski, 2009). There is, however, a general consensus for a slowing and increase in the power of both theta and delta oscillations, and a concurrent decrease in these parameters for the alpha and beta bands (Brenner et al., 1988; Coben et al., 1985; Giaquinto and Nolfe, 1986; Rae-Grant et al., 1987; Soininen et al., 1982; Kowalski et al., 2001). However, due to the heterogeneous mixture of patients used in these studies in terms of their disease stage/pathology, it is likely that this approach will produce confounding or mixed results. Additionally, all human studies have been performed using scalp EEG electrodes, which reflect the summation of cortical LFPs. This has led to the suggestion that these studies may be more beneficial in the detection of cortical dementia, rather than subcortical forms (Verma et al., 1987; Jeong et al., 2001).

As such, it is imperative that we develop our understanding of how EEG rhythms are affected by disease within regions of the brain that are directly affected by pathology during the early stages of AD, such as the HF. Secondly, the use of murine models of AD allow for the control and predictability of disease and
5.3. Age and disease related changes in the spectral power of spontaneous network oscillations

pathology progression. Therefore, in this study the incorporation of the 3xTgAD mouse allows us to determine how the power of various rhythms alter with age and AD-like pathology in the CA1 and subiculum of the HF.

In contrast to the majority of human studies, this report shows a general and earlier decrease in the power of both delta and theta rhythms, and increases in alpha and beta in the 3xTgAD when compared to age-matched control mice. Whilst these results are different to those found from cortical EEG studies in humans, they do agree with the limited number of studies conducted in mice (Wang et al., 2002; Rubio et al., 2012; Scott et al., 2012). So why are these results so different? One reason is thought to be due to slight differences in disease mechanisms. Changes in LFP patterns in human studies are due to neurodegenerative effects in cholinergic innervation. Due to the key contribution of cholinergic innervation to the generation of rhythmic oscillations within the HC, the subsequent progressive loss of innervation results in a loss of rhythmicity (Hangya et al., 2009; Apartis et al., 1998; Simon et al., 2006; Yoder and Pang, 2005; Schliebs and Arendt, 2006; Platt and Riedel, 2011). This cholinergic loss, however, is not typically observed in mice (Liu et al., 2002; Wong et al., 1999).

Whilst this represents one discrepancy between the mechanisms in human and AD mice, I observe alterations in another key regulator of network oscillations. Rhythmic GABAergic innervation is required for the generation and maintenance of various network oscillations, including theta and gamma (Buzsáki, 2002). Recent studies have implicated a change in inhibitory tone with age and disease progression, initially resulting in a state of hyperactivity followed by hypoactivity, and that this is typically pathology-dependant (Palop and Mucke, 2010b,a; Rubio et al., 2012; Davis et al., 2014). Furthermore, reductions in the complexity of GABAergic septo-hippocampal axons have been observed in AD, and a general increase in the release of GABA from activated astrocytes (Rubio et al., 2012; Jo et al., 2014). As such, it is likely that these disease-related changes in GABAergic tone will lead to direct alterations in the generation and maintenance of rhythmicity within the HF. Additionally, it is likely that changes in the monoaminergic system will contribute to the changes observed in LFP power. Although complex, the monoaminergic system contributes to the modulation of cortical and hippocampal oscillations, and therefore has an important role in attention and memory (see (Trillo et al., 2013) for a review). Human studies have indicated that there is a general reduction in monoaminergic tone with age, and other studies have shown that this system plays a key role in the modulation of state-dependant oscillations (Grudzien et al., 2007; Matthews et al., 2002; Kocsis et al., 2007; Scullion et al., 2011).

As a result, we do not currently understand the exact mechanisms involved in
the alterations of network oscillations observed in human and mouse AD. However, it is evident that there is a very complex and vast network changes associated with age and disease that requires further investigation. Whilst I demonstrate differences and similarities between other human and mouse studies, it is important to consider that this is one of the first studies to conduct *in vivo* multi-electrode sub-cortical recordings in regions of the brain directly affected by AD. It has been widely accepted that the 3xTgAD mouse develops AD-like pathology and cognitive deficits in a spatial and temporal manner similar to that observed in human AD. It is envisaged that the correlation between changes in cognition and the early alterations in network rhythmicity will provide disease-state specific benchmarks for evaluating the effectiveness of future treatment options.

### 5.4 Deficits in the information carrying capacity during spontaneous network oscillations

In addition to the assessment of synaptic connectivity and plasticity in the CA1-subicular pathway, I applied information theoretic principals in order to quantify the information carrying capacity during physiologically relevant spontaneous network oscillations. To date, most studies have focused on the assessment of functional connectivity between regions of the brain through the implementation of correlation and power analysis ([Brenner et al., 1988](#Brenner1988), [Coben et al., 1985](#Coben1985), [Giaquinto and Nolfe, 1986](#Giaquinto1986), [Rae-Grant et al., 1987](#Rae-Grant1987), [Soinin et al., 1982](#Soinin1982), [Kowalski et al., 2001](#Kowalski2001)). However, these have provided conflicting information, again probably due to the large distribution of patients with differing degrees of disease progression.

Whilst these studies provide an indication as to how interconnected regions are coherently paced with one another, they provide little insight into how information flows between them. In addition, they do not discount the influence of shared common inputs that may result in the synchronous activity between inter-connected regions ([Friston, 2011](#Friston2011)). In this study, I assessed effective connectivity, by investigating inter-regional connectivity through the implementation of transfer entropy analysis, which determines the causal influence of one system over another ([Shannon, 1948](#Shannon1948), [Granger, 1969](#Granger1969), [Schreiber, 2000](#Schreiber2000)).

In terms of causal influences, and in support of recent electrophysiology and anatomical data, I demonstrate that the subiculum does not merely act as an output region of the HC ([Deadwyler and Hampson, 2004](#Deadwyler2004), [Sun et al., 2014](#Sun2014), [Jackson et al., 2014](#Jackson2014)). Instead, I show substantial information flow in a bi-directional manner between CA1 and subiculum. This is of great importance, since the neuronal deficits
Deficits in the information carrying capacity during spontaneous network oscillations reported in this study do not only suggest that there is a breakdown in the output of information from the HF as would be proposed by the unidirectional trisynaptic loop hypothesis [Andersen et al., 1971], but would actually result in a significant reduction in hippocampal input.

In addition to early synaptic deficits reported in this study, I also show that there is a significant reduction in the information carrying capacity in the HF during early stages of AD, prior to overt pathology. I demonstrate that changes become apparent as early as 3 months, with deficits specific to the delta, and in particular to the higher frequency SWR component of the delta oscillation. This result correlates well with the development of cognitive and electrophysiological alterations, where 3xTgAD mice have impaired consolidation (but not acquisition) of spatial memory (Billings et al., 2005), and alterations in the hippocampal circuitry at 4-5 months of age (Davis et al., 2014). These delta/SWR deficits are likely to result in severe impairment of consolidation of hippocampal dependent memories, perhaps during SWS.

The HC is typically viewed as a memory buffer, where memories are initially created within the HF and later transferred to the neocortex for long-term storage (Squire 2002). In this respect, theta rhythms are involved in the acquisition of memories, and slow-wave/delta rhythms play a key role during the consolidation phase (Hasselmo et al., 2002; Born et al., 2006; Stickgold and Walker, 2005; Diekelmann and Born, 2010; Molle and Born, 2011; Walker and Stickgold, 2004). Specifically, in support of consolidation deficits and significant reductions in information within the delta/SWR band, research has shown that SWRs are a key component in the consolidation of memories during delta (non-REM sleep) oscillations (Sirotta et al., 2003; Girardreau et al., 2009; Ego-Stengel and Wilson, 2010; Buzsáki, 1989; Logothetis et al., 2012). Studies of cells involved in the formation of the cognitive map provide further evidence for the physiological role of SWR oscillations. Place cell activity is found to ‘replay’ during non-REM sleep, specifically during SWR events. In addition, the firing rate is typically enhanced when compared to the ‘online’ version of that map and, therefore, SWRs are key for the consolidation/reinforcement of spatial memory (Pavlides and Winson, 1989; Wilson and McNaughton, 1994; Buzsáki, 1989; Skaggs et al., 1996; Lee and Wilson, 2002). Furthermore, disruption of SWR activity has been found to correlate with severe impairments in memory consolidation (Girardreau et al., 2009; Ego-Stengel and Wilson, 2010). Therefore, it is now becoming evident that there are vast differences at both the synaptic and network level during the early/prodromal stages of AD. These early and state-dependent alterations prior to AD pathology suggest that there is a link between the development of intracellular and/or extracellular oligomeric forms Aβ and both cognitive and synaptic deficits (Palop and Mucke, 2010a; Palop et al., 2007; Oddo et al., 2003; Billings et al., 2005;
Our study further shows that network alterations are complex, and develop with both age and disease progression. At 6 months I detect differences in the information carrying capacity within the theta frequency band. Whilst this represents the slower, atropine-sensitive form of theta that exists under urethane anaesthesia, we still detect a robust change. Rhythmic activity is under tight control of excitatory and inhibitory inputs from a combination of intrinsic (EC/CA3) and extrinsic (medial-septal) pacemaker regions (Hangya et al., 2009; Vertes and Kocsis, 1997; Hasselmo, 2005; Buzsáki, 2002). As such, it is conceivable that even a slight change in modulatory control will alter the dynamics of the network. Due to the early (4-6m) breakdown in inhibitory control, leading to hyperactivity of the DG/CA1 regions of the HC, I propose that this may lead to disruption of the intrinsic control of this tightly regulated network and, therefore, accounts for at least some of the changes observed in the theta band at 6 months (Davis et al., 2014). Again, the differences I observe in network communication at 6 months correlates with the deficits observed during episodic-like memory tasks (Davis et al., 2013b,a). By 9 months, I show a slight difference in the slow-wave component of the delta signal, with both genotypes dropping to similar levels in the theta component. This correlates with behavioural studies indicating a drop in the cognitive capacity in both control and 3xTgAD mice (Davis et al., 2013b,a) and, therefore, is likely to reflect disease and age-related decline in cognition.

5.5 Limitations of the study

This thesis reports the first evidence of an early and significant breakdown in the flow of information and neuronal connection between the CA1 and subiculum in the 3xTgAD mouse. However, it is important to consider the limitations and caveats of the study.

Firstly, it is important to consider the validity of the use of animal models to evaluate the changes associated with a human disease. While we have very few alternative options for conducting preclinical studies, we should be cautious in directly comparing the pathophysiology of the disease in rodents versus humans. For example, the expression and development of AD-like pathology is under tight control and regulation of promoters in the rodent; a feature which is not known in the human form of the disease. In the 3xTgAD mouse, transgenics is under the control of the Thy 1.2 promoter, and therefore it is difficult to compare the expression and development of AD pathology to humans (Oddo et al., 2003; Braak and Braak, 1991). Additionally, it is unlikely that the pathophysiology of the disease
is comparable between models and humans. One major of the major limitations associated with the use of rodent models of AD is the lack of the development of tau pathology following the introduction of a combination of APP and/or PS1/PS2 transgenes. However, the recent development of a rat model has shown promising results, with the introduction of APP and PS1 transgenes results in the development of tau pathology in a similar spatiotemporal manner as that observed in human AD (Cohen et al., 2013). Therefore, while there are limitations associated with the use of the 3xTgAD mouse, studies have shown that the model develops similar cognitive deficits to those observed in human patients. Further, this and other studies have reported neuronal deficits that are in line with the cognitive deficits (Oddo et al., 2003; Billings et al., 2005; Davis et al., 2014) and therefore validates the use of murine models in preclinical studies.

Another limitation of this study is the difficulty in determining and correlating the underlying pathology with the onset and progression of cognitive and neuronal changes. Recently, studies have indicated that AD-related changes are associated with an increase in intracellular/soluble forms of Aβ rather than with Aβ plaques (Klyubin et al., 2012; Oddo et al., 2003; Billings et al., 2005). However, studies have failed to reliably dissociate between intracellular, soluble and insoluble Aβ pathology. For example, Oddo et al. (2003) measured soluble forms of AB through the use of ELISA, but do not report if formic acid was used in their preparation; a method which is known to influence the detection of insoluble/soluble forms of Aβ, and therefore could lead to misinterpretation (D’Andrea et al., 2003). Additionally, the A11 Aβ antibody was used to detect oligomeric forms of Aβ in a study by Oddo et al. (2003), but fail to show evidence of a negative control. Therefore, we cannot account for the specificity of the antibody used.

Importantly, intracellular forms of Aβ have not been reported in human cases of AD. The amyloid cascade pathway hypothesises that AB generation is predominately of an extracellular pathway, and so the proposed mechanism of production seems to be contradictory to the build of intracellular Aβ reported in some studies (Hardy and Higgins, 1992). However, it is important to consider that human tissue is typically evaluated post-mortem, when the patient is at an advanced stage of the disease. Therefore, it may well be that the soluble/intracellular forms of Aβ have not yet been reported/detected in humans.

It is also important to consider the limitation associated with the use of an anaesthetised preparation to assess changes during different network oscillations. For example, during urethane anaesthesia, there is the loss of the faster atropine-insensitive theta oscillation that is observed in the behaving animal (Kramis et al., 1975; Clement et al., 2008). Additionally, studies have recently demonstrated the
dynamics and control of GABAergic interneurons are state- and behaviour-dependent (Lapray et al., 2012; Varga et al., 2012; Katona et al., 2014; Varga et al., 2014). Therefore, the conclusions of the study may be specific to the anaesthetised state and may not be directly translatable to the awake, behaving animal. However, it is important to consider that while we appreciate that there are differences between the anaesthetised and awake animal, we detected strong and measurable differences between the control and diseased animal, and therefore adds to the understanding of the changes that occur with AD-like pathology in the 3xTgAD mouse.

During urethane anaesthesia, we observed a concurrent peak in the delta frequency band during theta rhythms. To the best of our knowledge, this has not been previously reported, and therefore it is possible that this may in fact be a breathing artefact. However, this is not likely to have influenced the outcome of the analyses as the temporal pattern of a breathing artefact would be identical between the regions of interest and is therefore unlikely to result in the emergence of genotypic differences.

Finally, we propose that the information deficit observed during delta-dominant periods is attributed to a specific deficit in SWRs. However, this conclusion is based on SWR detection by band-pass filtering alone. A recent study by Scheffer-Teixeira et al. (2013) demonstrated that high-frequency oscillations >100Hz can be produced by spike-leaked high-frequency oscillations. As such, we cannot discount for this contributing to our findings, but it is unlikely to result in such robust genotypic differences as observed in this study. Therefore, to validate this finding, future work should use a number of SWR detection methods, such as the reversal of SWRs across the ventral-dorsal axis (Scheffer-Teixeira et al. 2013).

5.6 Conclusions and future work

This study is the first of its kind, conducting a thorough in vivo investigation of the synaptic and network changes associated with age and AD within CA1 and subiculum. With only one other in vivo and in vitro study conducted in the most comprehensive mouse model of AD (the 3xTgAD mouse), this work progresses and develops our understanding of the changes associated with the disease (Oddo et al., 2003; Davis et al., 2014).

Recent work has demonstrated deficits in a hippocampal-dependant episodic-like memory task, correlating with hyperexcitability in the CA1/DG network, prior to the development of overt pathology. In contrast, this thesis demonstrates early and significant reductions in neuronal excitability and complex changes in the information...
carrying capacity of the CA1-subiculum pathway. As such, the most recent data obtained from this and other work reveals that the pattern of synaptic alterations are much more complex than originally thought, with hypoexcitability within the subiculum at an age when hyperexcitability is observed in the PP input to DG/CA1. As such, it is now clear that the HF is not uniformly affected by AD-like pathology; rather, there are complex regional changes. Therefore, this new finding is likely to impact future research of potential therapeutics. It is likely that the impact of therapeutics on hippocampal circuitry will depend on the stage of disease progression or the region being assessed. Additionally, I propose that late-stage AD is similar to an advanced ageing phenotype. In this study, and in agreement with others, I observe a general age-related decrease in synaptic facilitation, and a reduction in information carrying capacity (Disterhoft and Oh [2007]). By 9 months, synaptic facilitation during PPF and repetitive LFS decreases to similar levels to those observed in 3 month 3xTgAD mice. I also show a lack of detectable differences in the information carrying capacity of network oscillations at this age. By 9 months there are slight genotypic differences evident in the slow-wave component of the delta signal. Additionally, this report reveals that by 9 months, the information carrying capacity of the theta signal drops to similar levels in both genotypes, and therefore may account, at least to some extent, for the changes in cognition observed in both control and 3xTgAD mice. By this age, control and 3xTgAD mice display evidence of reduced cognitive capacity. Additionally, our evoked electrophysiological study demonstrates that aged control mice suffer from an age-related decrease in synaptic performance; reaching similar levels as those observed in the 3xTgAD mice at 3 months. Therefore, this report demonstrates an age- and AD-like pathology related change in neuronal communication. My results demonstrate a robust pattern, where 3xTgAD mice undergo advanced and early changes in neuronal communication/connectivity, with control mice displaying similar alterations as those observed in the 3xTgAD mice as a function of age. Therefore, the 3xTgAD mice display an advanced ageing-like phenotype.

Further work will be required to further investigate the mechanisms underlying the discrepancies between some of the changes observed in human vs mouse models of AD. The present work has demonstrated for the first time that there are early changes in oscillatory activity as determined through power and causal analysis, a protocol that could, in theory, be applied to human EEG recordings. This work provides further support for the development of EEG tools in the research and diagnosis of AD.

However, this work demonstrates some of the neuronal changes associated with disease in only one particular model of AD. Given more time, it would be ideal to compare the results obtained in this study with recordings from other mouse and rat
models. Specifically, the effect of the tau transgene should be investigated further, and therefore comparisons could be made with APP/PS1 models such as the TASTPM mouse. Whilst I do not observe tau pathology at any of the ages used within this study, I cannot discount for an effect of the introduction of the tau transgene. As discussed earlier, a recent rat APP/PS1 model of AD has been produced (Cohen et al., 2013). This model is unique in that it spontaneously develops tau pathology without the introduction of tau genes, together with significant cell loss. This model develops pathology and cognitive deficits from 6 months, with substantial pathology evident by 15 months. However, there has been limited behavioural characterisation and no electrophysiological studies to date. Due to the combination of the closer genetic link between rats and humans, and the closer AD phenotype of the model, it is likely that this could result in greater translational prospects (Cohen et al., 2013).

In addition, this and other work confirms that the HF is not affected by disease and pathology in a uniform manner, and as such there are complex and opposing alterations within the circuitry. It is therefore vital to develop our understanding of how synaptic excitability changes evolve across the entire HF, and how this alters with age. For example, it is known that the PP to DG/CA1 displays hyperexcitability at the same time that the CA1-subiculum pathway expresses hypexcitability (Davis et al., 2014). Other work has shown that there is synaptic depression in CA3-CA1 pathway, albeit in a reduced in vitro preparation (Oddo et al., 2003). However, to the best of my knowledge, I am unaware of any electrophysiological recordings that have assessed synaptic changes in the EC. It is assumed that, during repetitive subicular stimulation, neuronal activity propagates from deep to superficial EC layers and then re-enters DG/CA1 regions as a reverberatory wave of activity (Davis et al., 2014; Kloosterman et al., 2003, 2004). However, further work is required to investigate the synaptic changes occurring within this key region of the HF. Acting as the interface between hippocampal input and output, degeneration/synaptic deficits within EC would most likely have substantial implications in the physiology of memory.

We know that one of the principal cognitive functions of the HF is navigation and that HF regions contain spatially selective cells that serve to encode an internal representation of the external environment, developing the cognitive map and supporting path integration (O’Keefe and Dostrovsky, 1971; Ekstrom et al., 2003; Leutgeb and Mizumori, 1999; McNaughton et al., 2006). However, limited work has been conducted on determining the changes that occur in these spatially sensitive cells with age and disease progression. With only two studies conducted to date, there is a paucity in our understanding of how the neuronal changes observed in synaptic facilitation/connectivity impact both navigation and the encoding of declarative memories (Cacucci et al., 2008; Cheng and Ji, 2013). Studies have indicated that
3xTgAD mice have impaired spatial memory at 4 months, however, we do not know if this represents a reduction in the ability of the system to develop its cognitive map. Is there a lack of place/location encoding? Or is there a reduction in the ability to encode path integration? These are both questions that should be addressed in the future.

Whilst the results in this thesis are the first to investigate and report the network changes within the CA1 and subiculum associated with AD-like pathology progression, it is important to consider the limitations of using anaesthetised preparations. This study represents one of only a few that has investigated the impact of AD-like pathology in vivo, however, whilst this is clearly an advantage to traditional in vitro preparations, it would be advantageous to conduct future recordings in the awake animal. Under urethane-induced anaesthesia, we are able to investigate the neuronal dynamics during REM and non-REM-like network oscillations, however, we are yet to fully understand the exact mechanisms involved in anaesthesia, and therefore these results act to provide an indication into what may occur during ‘normal’ physiological wake and sleep states. Furthermore, the use of chronically implanted preparations will allow for the assessment of neuronal function and pathology-related changes during a variety of behavioural testing paradigms. This would allow for the direct correlation between alterations in the hippocampal network connectivity with the onset of cognitive changes. Further investigation into the use of novel therapeutics in the treatment of AD could be investigated, with a direct correlation of the effectiveness of the drug with changes in cognition, network connectivity and AD-associated pathology.
References


References


References


