Dissociating Aberrant Properties of Recognition Memory in the Tc1 Mouse Model of Trisomy-21

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Jessica Helen Hall

School of Psychology, Cardiff University

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"When the work you've put in is realised, let yourself feel the pride but always stay humble and kind." ~ Lori McKenna

Thesis Summary

Down's syndrome is a complex genetic condition arising from trisomy of chromosome 21; it is characterised by alterations in behaviour and synaptic plasticity, leading to deficits in learning and memory. The Tc1 mouse is a transchromosomic mouse model of trisomy-21 which carries a freely segregating and almost complete human chromosome 21. The main aim of this thesis was to explore recognition memory processes in the Tc1 mouse model of trisomy-21.

The initial goal was to provide insight into the learning and memory changes associated with triplication of genes on human chromosome 21 in mice. The Tc1 mouse had previously been shown to display a deficit in object recognition memory following a delay of 10-min, but not following a 24-h delay. This thesis aimed to confirm and extend these findings, by testing Tc1 mice on an array of novelty based recognition tasks. This thesis also aimed to explore some of the biological systems underpinning the pattern of learning and memory changes demonstrated by the Tc1 mouse. The expression of *c- fos* was used as a marker of neuronal activity, allowing for the assessment of regional activity, and in addition, the expression profile of the GluR1 subunit of the AMPA receptor and the GluK1 and GluK5 subunits of the Kainate receptor were examined. Finally, this thesis investigated the impact of the administration of a novel AMPAkine, drug 9A, on the cognitive phenotype of the Tc1 mouse.

The behavioural and biochemical analyses provided evidence that the Tc1 mouse model showed a selective deficit in short-term recognition memory while sparing long-term memory for the same type of information, and that a near complete copy of human chromosome 21 in Tc1 mice did not impair place recognition. In addition, *c-fos* expression studies provided evidence for aberrant perirhinal cortex activity in response to familiarity. Further to this, there was a significant reduction in expression of the GluR1 AMPA receptor subunit in the hippocampus, and a significant reduction of the GluK5 kainate receptor subunit expression in the perirhinal cortex. Finally, this thesis provides some preliminary evidence that a novel positive allosteric modulator, drug 9a, had a positive cognitive enhancing effect in the Tc1 mouse.

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List of Abbreviations

A1	Primary active
A2	Secondary active
AD	Alzheimer's Disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of Variance
APP	Amyloid Precursor Protein
ASVD	Atrioventricular Septal Defects
ASDII	Secundum Atrial Septal Defect
AW1 and AW2	Guanidine hydrochloride
BSA	Bovine Serum Albumin
CA	Cornus Ammonis
CANTAB	Cambridge Neuropsychological Test Automated Battery
CHD	Congenital Heart Defects
CNQX	6-cyano-7-nitroquinoxaline-2, 3-dione
CS	Conditioned Stimulus
DAB	3-3'-diaminobenzine
ddH_2O	Double distilled water
DG	Dentate Gyrus
DNA	Deoxyribonucleic Acid
DPX	Di-n-butyl phthalate
DR	Discrimination Ratio
DS	Down Syndrome
DSCR	Down Syndrome Critical Region
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalogram

ELISA	Enzyme-linked immunosorbent assay
EPSP	Excitatory Post Synaptic Potential
ES	Embryonic Stem
GABA	Gamma aminobutyric acid
HD	Hirschsprung's Disease
Hsa21	Human Chromosome 21
I	Inactive
IEG	Immediate Early Gene
LTD	Long Term Depression
LTP	Long Term Potentiation
Mmu	Mouse Chromosome
mPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
MSEL	Mullen's Scale of Early Learning
MTL	Medial Temporal Lobe
MWM	Morris Water Maze
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione
NGS	Normal Goat Serum
NMDA	N-methyl-D-aspartate
NOR	Novel Object Recognition
SNX-27	Sorting nexin 27
SOP	Sometimes Opponent Process
TAE	Tris Acetate
TBE	Tris Borate EDTA
TG	Transgenic
TOF	Tetralogy of Fallow
Ts	Trisomy
TTS	Triple Trisomic Mice

PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
PFC	Prefrontal Cortex
Prh	Perirhinal Cortex
ROI	Region of Interest
US	Unconditioned Stimulus
WCST	Wisconsin Card Sorting Task
WM	Working Memory
WT	Wild Type

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1. Introduction

This introductory chapter will initially outline the history, prevalence, aetiology and symptoms of Down syndrome (DS), before commencing with a more in depth discussion of the neurological and behavioural phenotype seen in human individuals with the syndrome. Numerous mouse models of trisomy-21 are available, each with their own advantages and disadvantages. The available mouse models will be discussed; first the genetic differences between the models will be noted, before a more detailed description of the cognitive phenotypes of the mouse models is given, including changes in synaptic plasticity, and behavioural phenotypes. Finally a more detailed description of the Tc1 mouse model, the model that is the focus of this thesis, will be provided, before the aims and hypotheses of the thesis are described.

1.1. Down syndrome

1.1.1. History of DS

Down syndrome (DS) was first described by John Langdon Down in 1886, who identified a group of patients with similar characteristics, including a flat, broad face, thick lips, a fissured tongue, and upward slanting eyes (Down, 1886). Down referred to these patients as “mongoloids”, based on his notion that these children looked like people from Mongolia. His paper “observations on an ethnic classification of idiots”, though ethnically inaccurate, is historically interesting in that it was the first clinical description of DS. In the 1960’s the term “mongolism” was heavily criticised, and as a result the condition was renamed “Down’s syndrome” after the very physician who first described the symptoms. Down had previously noted that symptoms were congenital, but misinterpreted the cause as being tuberculosis in the parents (Down, 1886). The actual genetic cause was not documented until 1959 by Jerome Lejeune’s group in Paris, who discovered that trisomy of chromosome-21 is the cause of DS (Lejeune, Gautier, & Turpin, 1959).

1.1.2. Prevalence of DS

DS occurs in 1 in 1000 births (Wu & Morris, 2013a), a decrease when compared to a rate of 2.3 in 1000 live births in 1945 (Wu & Morris, 2013b). It is thought that despite an increase in maternal age, increased prenatal diagnosis of DS and thus subsequent terminations, means that the live birth prevalence of DS has remained fairly stable since the introduction of prenatal tests in the 1980's (Wu & Morris, 2013b). In the 1950's, the rate of survival of DS live births was poor, with only 57% living past 1 year of age; survival has now increased to 90% (Wu & Morris, 2013a, 2013b). It is estimated that there were 37, 090 people living with DS in England and Wales in 2011, a population prevalence of 0.66 in 1000 (Wu & Morris, 2013a). Life expectancy of individuals with DS is currently averaged at 51 years of age, which although a huge success, also brings added complications which make it paramount that we find ways of tackling some of the clinical symptoms of DS.

1.1.3. Genetics of DS

1.1.3.1. Trisomy-21

Down syndrome is a chromosomal abnormality, which is characterized by the presence of an extra copy of human chromosome 21 (trisomy-21); the disorder is considered one of gene dosage, whereby the resultant overexpression of the genes on chromosome-21 are responsible for the DS phenotype (Hernandez & Fisher, 1996). Approximately 90-95% of individuals with DS have 47 chromosomes as opposed to the 46 which are present in the typical population. The remaining 5% are caused by translocation, or are mosaic or partial trisomy (Desai, 1997). A major advance in the study of DS was made by Penrose, by demonstrating that maternal age correlated with occurrence of DS (Penrose, 1933) and it follows that the greatest associated risk factor for having a child with DS is increased maternal age (Loane et al., 2013). Paternal age on the other hand has been found to be an insignificant factor (Gaulden, 1992). The most common cause of trisomy 21 lies with non-disjunction in meiosis, whereby chromosome 21 fails to separate during egg or sperm development; approximately 95% of cases result from nondisjunction in the maternal gamete, and 5% in the paternal gamete (Antonarakis, 1991).

1.1.3.2. Translocation DS

Less commonly, Down syndrome can also occur due to a Robertsonian translocation. Robertsonian translocations occur when there is end-to-end fusion of two acrocentric chromosomes; individuals with translocation Down syndrome have two normal copies of chromosome 21, plus some extra material from chromosome 21 attached to another chromosome (Robinson et al., 1994). Translocation DS is often referred to as familial DS, and accounts for approximately 4% of DS cases (Jyothy et al., 2001). Depending on whether the chromosomal aberration is balanced or not, Robertsonian translocation can result in a child with Down syndrome, a silent Robertsonian translocation carrier, or an unaffected child (Sudha & Gopinath, 1990). Silent carriers of Robertsonian translocations involving chromosome 21, and any of the acrocentric chromosomes, namely 13q, 14q, 15q and 22q, face a high risk of spontaneous miscarriage, or giving birth to either a child with Down syndrome or to another carrier (see figure 1.1) (Kolgeci et al., 2015).

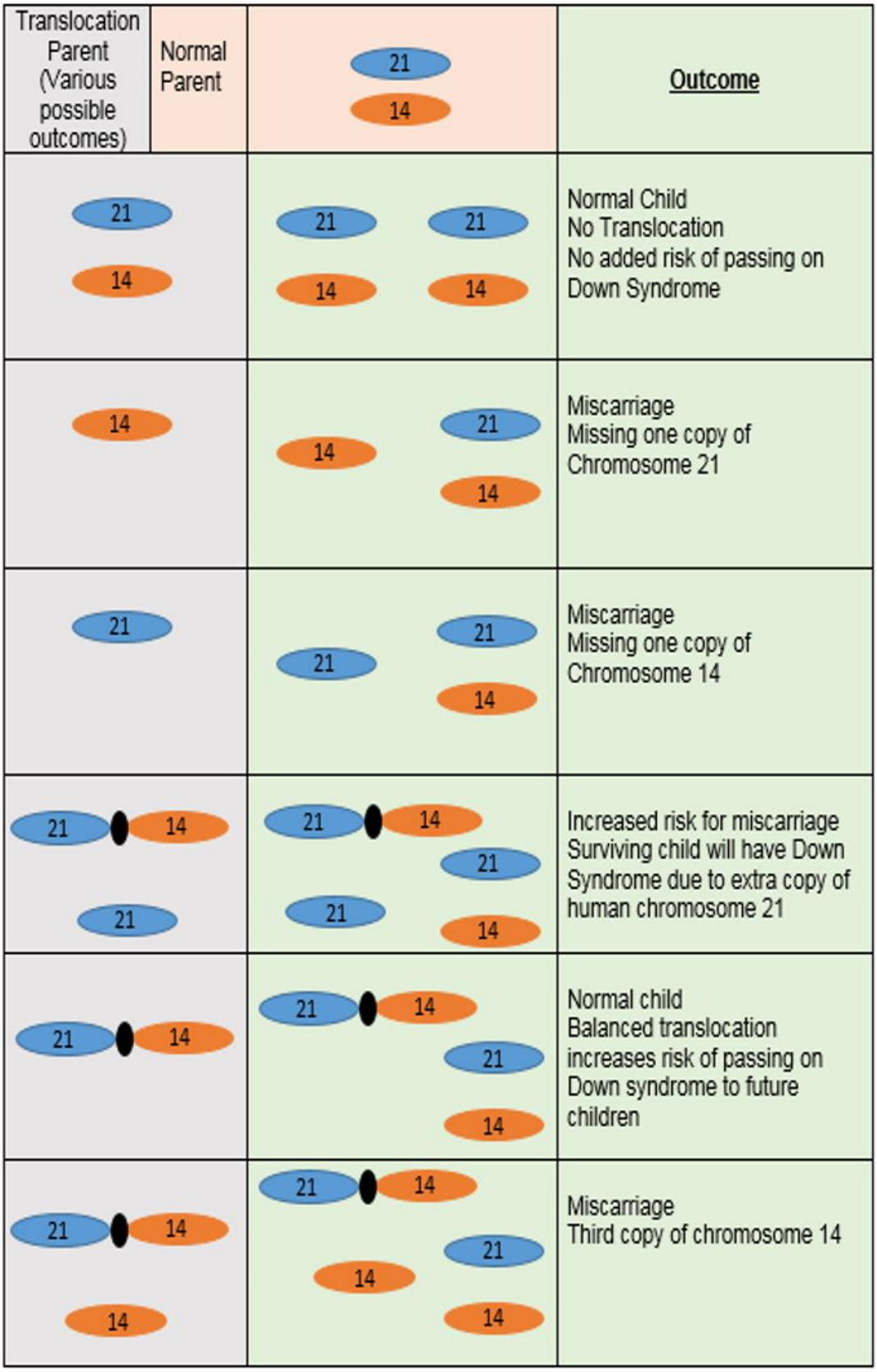


Figure 1.1 *- Figure depicting the possible outcomes of Translocation DS.* The normal parental gamete is illustrated, with one copy of chromosome 21, and one copy of chromosome 14. The left hand column depicts all the possible combinations of a translocation parental gamete. The central column depicts the genetic outcome of the resulting zygote.

1.1.3.3. Mosaicism

The remaining cases of DS are mosaic; mosaicism is a condition which occurs when an individual has two or more genetically distinct cell lines which develop from a single zygote (Papavassiliou et al., 2009). Thus, in mosaic cases of DS, some cells in the body are euploid and have the typical 46 chromosomes, but some cells in the body are trisomic for chromosome 21 and thus have 47 chromosomes (Papavassiliou et al., 2009). It has been demonstrated that the mechanism resulting in mosaicism is meiotic in 60% of cases, whereby the initial zygote has three copies of chromosome 21, which normally would result in simple trisomy 21, but during the course of cell division one or more cell lines lose one of the chromosomes (Pangalos et al., 1994). The remaining 40% of cases are mitotic, whereby the initial zygote has two copies of chromosome 21, but during the course of cell division one of chromosomes is duplicated (Pangalos et al., 1994). Individuals with mosaic DS show a broad range of phenotypes, but are generally higher functioning than those with full trisomy (Johnson & Abelson, 1969). A study assessing 30 subjects with trisomy 21 and comparing them to 30 age, sex and socioeconomic status matched individuals with mosaic DS, found that the mean IQ of the mosaic group was significantly higher than that of the group with complete trisomy (Fishler & Koch, 1991). More specifically, it has been determined that a variation in phenotype is associated with the percentage of trisomic cells (Papavassiliou et al., 2009). When split into two distinct classes, those in the class with the lower percentage of trisomic cells, showed significantly fewer DS phenotypic traits than those in the class with the higher percentage of trisomic cells.

1.1.3.4. Partial Trisomy

Occasionally, just a portion of chromosome 21 will undergo duplication, and this results in partial trisomy. It has been argued that the major phenotypic features of the syndrome, including mental retardation, congenital heart defects, and the characteristic facial features, are consistently present even if the partial trisomy only includes the distal arm of chromosome 21; band q22 (Korenberg et al., 1990). These kinds of rare partial trisomy, which display many of the phenotypes associated with DS, have resulted in many researchers believing that there is a DS 'critical region' on chromosome 21, and that trisomy of this region is causal of the syndrome (McCormick et al., 1989). Larger studies have used cases of partial trisomy to map the genomic regions which are associated with specific phenotypes, arguing against the DSCR and highlighting the heterogeneity of each case (Lyle et al., 2009).

1.1.4. The DS phenotype

Trisomy 21 causes an array of phenotypes, with craniofacial abnormalities, muscle hypotonia and mental retardation being the most consistent of DS features, being conserved in 100% of individuals with DS (Hernandez & Fisher, 1996). Individuals with DS may also be affected by other phenotypes which show variable expressivity in the DS population. Phenotypic features of Down syndrome which are physically visible include brachycephaly, brachydactyly, broad hands, epicanthal folds, a flat nasal bridge, lax ligaments, a short stature and an open mouth (Roizen & Patterson, 2003). In addition, individuals with DS can also experience other clinical symptoms to varying degrees, including heart defects, gastrointestinal problems, haematological abnormalities and audiological and visual difficulties (Asim, Kumar, Muthuswamy, Jain, & Agarwal, 2015).

1.1.4.1. Cardiac Defects

Approximately 50% of children with Down syndrome are born with CHD, and thus all newborns with Down syndrome are screened for lesions (Asim et al., 2015). The defect tends to be caused by abnormal development of the endocardial cushions, and the most common defects are atrioventricular septal defects (AVSD), which is caused by the failure of the endocardial cushions to come together during embryonic development, resulting in a defective opening between the atria and the ventricle of the heart (Wilson et al., 1993). Individuals with DS account for 70% of AVSD cases, highlighting the specificity of AVSD within trisomy 21, and suggesting that chromosome 21 may be a candidate for many of the genes involved in effective embryonic separation of the atria and ventricles (Barlow et al., 2001). Other common CHD features include secundum atrial septal defect (ASDII), ventricular septal defect (VSD), and tetralogy of Fallot (TOF) (Freeman et al., 2008).

1.1.4.2. Gastrointestinal Problems

Hischsprung disease (HD) is a disease of the large intestine which is caused by a lack of ganglion cells in a length of the colon (Amiel et al., 2008). HD causes failure of the distal intestine to relax properly, meaning that peristaltic waves do not occur properly in order to push excrement along the length of the intestine, ultimately resulting in intestinal obstruction and thus, severe constipation (Amiel et al., 2008). DS individuals account for 12% of HD cases (Asim et al., 2015), this association again suggesting that genes which are responsible for susceptibility to HD may map to chromosome 21.

1.1.4.3. Childhood Leukaemia

Individuals with DS have a greatly increased risk of leukaemia, with studies showing up to a 20 fold increase in risk of leukaemia in children (Hasle, Clemmensen, & Mikkelsen, 2000). Individuals with DS account for 10% of all cases of childhood leukaemia; leukemogenesis in patients with Down syndrome is associated with the presence of somatic mutations involving the *GATA1* gene (Xavier & Taub, 2010).

1.1.4.4. Other clinical problems

There are numerous additional problems which can occur in individuals with DS, for example, congenital cateracts develop in a high proportion of DS individuals (Haargaard & Fledelius, 2006) and congenital loss of hearing in DS neonates is considerably higher at 15% than the 0.25% of cases in typically developing children (Tedeschi et al., 2015). Both hearing and vision are as a result monitored from birth. There is also high incidence of coeliac disease, insulin dependent diabetes, and hypothyroidism, which warrants routine screening in all Down syndrome infants (Glastras et al., 2005). Medical professionals are also vigilant about the increased prevalence of epileptic seizures (Asim et al., 2015).

1.1.4.5. Neurological differences in DS

Down syndrome is the most common genetically defined cause of intellectual disability, with individuals experiencing cognitive impairments, including deficits in learning and memory (Silverman, 2007). The mental retardation experienced in Down syndrome is one of the most conserved features across the disorder, and typically consists of developmental delays, learning and memory deficits and difficulties with verbal processing (Hernandez & Fisher, 1996). IQ tends to average at around 50, with a range of 20-70; this reflects individual differences but also emphasises the conserved cognitive phenotype (Chapman & Hesketh, 2000). Although IQ is generally low in DS individuals, other genetic and environmental factors also impact IQ in the same way it would in the typically developing population; there is a positive correlation between parental IQ and the IQ of individuals with DS (Pennington, Moon, Edgin, Stedron, & Nadel, 2003). Cognitive development is considered to lag behind that of typically developing children. A series of longitudinal studies monitoring cognitive progress from birth to 11 years of age showed cognitive development to be slower in children with DS (Wishart, 2016). Subjects were seated securely in a position whereby a 60° foot kick would break a light beam, producing a 1-s rotation of a brightly coloured mobile. Non-delayed infants can detect this sort of contingency by at least 2 months of age, and kicking rate typically rises,

often accompanied by smiling or excited vocalizations. DS subjects were generally slower to detect this contingency than chronologically age matched controls. Interestingly, DS subjects with increasing age, seemed increasingly content to accept the lower levels of non-contingent reward being provided by the computer over the higher levels potentially available if they exerted their own control over the mobile's movements, suggesting an inability in maintaining existing skills due to indifference. Similarly when carrying out an object search task, despite initial acquisition of the task being similar to that of controls, DS subjects generally failed to maintain these successes longitudinally (Wishart, 2016). Analysis of behaviour during the test indicated low task engagement and high task avoidance (Wishart, 2016). As a result of the severe and consistent cognitive phenotype, the central nervous system is a major research focus in DS.

1.1.4.6. Morphological changes

On a gross anatomical level, magnetic resonance imaging (MRI) studies on live individuals have demonstrated clear differences in the brain morphology of individuals with DS when compared with control brains. However, the interpretation of some MRI studies of the brain in DS can be confounded by the inclusion of individuals with Alzheimer's disease, which may result in a confounding pathological profile which is not true to DS alone. In general however, a decreased frontal volume and normal thalamic volume in Down syndrome is apparent. A study by Jernigan, Bellugi, Sowell, Doherty, and Hesselink (1993) reported distinct patterns of brain morphology when scanning the brains of six adolescent DS individuals compared with controls. Cerebral volume was significantly reduced, and the proportional sizes of the anterior cortex and temporal limbic cortex were diminished. Additional studies have also gone on to support these findings. When measuring a number of brain regions in 25 subjects (13 DS, 12 age and gender matched controls), a study found that DS subjects had substantially smaller cerebral and cerebellar hemispheres, ventral pons, mammillary bodies, and hippocampal formations; the parahippocampal gyrus was larger in DS subjects (Raz et al., 1995). A similar study using 50 DS individuals and 23 controls demonstrated that total brain and gray-matter volumes were reduced in DS, as were the volumes of the left hippocampus and amygdala (Pearlson et al., 1998).

Consistent with the reduction in cortical volume, there is evidence for a reduction in the pace of dendritic maturation is significantly reduced in Down syndrome. In infancy, the normal dendritic tree continuously expands (Becker, Mito, Takashima & Onodera, 1991). This dendritic expansion appears normal in DS up to four months of age. However, after this period during the first year of development, the dendrites stop growing and become atrophic compared to control neurons (Becker, Mito, Takashima, Onodera, & Friend,

1993). When examining children aged 0 – 5 years, it is clear that there are also changes in neuronal organisation and number including around 20-25% fewer neurons, and unusual distribution of neurons, particularly in cortical layers II and IV (Wisniewski, 1990). Golden and Hyman (1994) hypothesised that this disorganisation of the cortical layers occurs during foetal development. Stereological cell counts demonstrate that until gestational week 21, neuronal migration in DS appears normal, however following this, the second stage of cortical development is delayed and disorganised, meaning that cortical layers are not properly formed (Golden & Hyman, 1994). Myelination delay was also observed in a much higher proportion of DS children (22.5%) than in the non-DS children (6.8%), most likely resulting in a lack of propagation of action potentials along the length of axons (Wisniewski, 1990). More recently, Abraham et al., (2012) studied the sequence of myelination in patients with DS longitudinally from in utero to adulthood. It was found that despite normal initiation of myelination, the number and density of myelinated fibres was significantly reduced, with subtle differences early on that increased across age. A study by Guidi et al., (2008) also reported early alterations to the MTL, including altered neurogenesis and reduced cell number in the hippocampus, dentate gyrus, and parahippocampal gyrus between 17 and 21 weeks gestation. These neuronal changes may represent some of the underlying causes of impaired cognition in DS.

1.1.5. The cognitive phenotype

There is a general consensus that DS affects 'late developing' neural systems, including the hippocampus and the prefrontal cortex (Edgin, 2013). Functional brain studies have frequently pointed towards reduced activity in the brains of individuals with DS, particularly in the regions associated with memory and language. For example, when performing a semantic classification task, whereby participants had to state whether a pictured animal lived on a farm, the activation of the brains of DS patients was markedly different to that of age matched controls; for example, in typically developing controls, the semantic classification task activated the primary visual cortex, lateral occipital cortex, the inferior and middle temporal gyrus, and portions of the lingual and fusiform gyri (Jacola et al., 2011). In contrast, the bulk of activation in DS individuals was seen in the frontal cortex (Jacola et al., 2011). Thus, some brain regions which are normally active in specific types of task performance are not recruited in DS, whereas other regions are overly active. With regards to the developmental phenotype of the DS brain, MRI studies have demonstrated reduced grey matter density and reduced dendritic branching, particularly leading to reduced hippocampal and frontal cortex volumes (Jernigan et al., 1993). Ultimately, it is evident that there seem to be changes in the prefrontal cortex, and the hippocampus of individuals with DS.

1.1.5.1. Speech and Language function

Language problems are a central feature of DS, and as a result speech and language are one of the best characterized elements of the cognitive profile in DS. Children with DS are slow to reach developmental verbal milestones, with slower progression from babbling to the formation of words (Pennington et al., 2003). Many different areas of speech and language development lag behind that of typically developing children. Individuals with DS have difficulty with articulation, utterance and vocal imitation (Fowler, Kile, & Hecox, 2001). In addition, vocabulary development is also poor; though the production of first words is similar to typically developing children. Nevertheless, the acquisition of new words is considerably slower (Berglund, Eriksson, & Johansson, 2001). A vocabulary spurt will often occur in children around the age of 18 months, this phenomenon occurs much later in children with DS, from around 30 months of age, to as late as five or six years of age (Galeote, Soto, Checa, Gomez, & Lamela, 2008).

Verbal short term memory is also deficient in DS, when compared to mentally age matched controls. This verbal short term memory deficit is seen consistently, and may explain some of the difficulties observed in DS with regards to vocabulary development. A typical measure of short term memory is the word- span test, in which participants are

presented with a list of verbal items that they have to immediately repeat. An individual's verbal short-term memory span is the maximum number of items that he or she can correctly repeat under these conditions; adults typically have word spans of around 7, plus or minus two (Miller, 1994). The verbal short-term memory spans of individuals with Down syndrome tend to be considerably lower. A study which assessed a group of 45 children and adolescents with Down syndrome for example found that the mean word span for this group was just 3.5 words (Bird & Chapman, 1994). Ultimately, the verbal short term memory problems which are evident in DS, are likely to contribute to a reduced vocabulary. Comprehending syntactic relations would require temporary memory storage of words. In turn, considering that a large proportion of IQ tests are based on linguistic ability and verbal comprehension, verbal short term memory deficits in DS are likely contributing to the compounded IQ scores seen in the syndrome.

One possible explanation for poor verbal memory, is that individuals with DS also experience significant auditory difficulties; hearing loss is particularly common in DS (Marcell & Cohen, 1992). It would thereby follow that if individuals were to have difficulty in hearing the spoken word, they would be bound to have difficulty processing, and subsequently recalling it. This has been assessed by contrasting memory auditory material with that for material presented in both the auditory and visual domains, to alleviate the effects of auditory difficulties. Participants with DS demonstrated verbal recall which was equally deficient when auditory material was presented, and when it was presented in both auditory and visual domains (Jarrold, Baddeley, & Phillips, 2002). It has thereby been suggested that a lack of rehearsal, or a deficit in the phonological loop of DS individuals may be at the root of language related problems. Data has indicated that DS children are significantly more accurate and intelligible when reading words aloud, than when they produce words themselves in a naming task (Knight, Kurtz, & Georgiadou, 2015), pointing towards reading as a positive facilitator in language development for DS children.

1.1.5.2. Medial Temporal Lobe Function

The Medial Temporal Lobe (MTL) is a system of anatomically linked structures which are essential for learning and memory. The system consists of the hippocampus, and the adjacent perirhinal, entorhinal, and parahippocampal cortices. The medial temporal lobe plays a major role in recognition memory; it is generally acknowledged that a major contribution of the hippocampus to recognition memory is processing object location and context information (Barker & Warburton, 2011; Wan, Aggleton and Brown, 1999; Duva et al., 1997, Jackson-Smith, Kesner & Chiba, 1993; Aggleton, Hunt & Rawlins, 1986). In contrast, there is relatively little evidence that the hippocampus contributes to short-term object memory (Hammond, Tull, & Stackman, 2004). Simply put, the dorsal and ventral visual streams converge onto the hippocampus, with the dorsal stream providing spatial information via the parahippocampal cortex, and the ventral stream providing object information via the perirhinal cortex (Kravitz, Saleem, Baker & Mishkin, 2011) (see Figure 1.2).

Individuals with DS are impaired on numerous tasks which map onto the MTL (Edgin, Mason, Spano, Fernandez, & Nadel, 2012), and given the complex connections of the MTL, these deficits may result from the dysfunction of the hippocampus itself, or from dysfunction of the pathways which converge onto the hippocampus (Edgin, Mason, Spano, Fernandez, & Nadel, 2012). Many studies have considered various tasks of verbal memory, however, these are often confounded by the fact that language function in DS is not comparable to that of typically developing individuals. This section will therefore focus on non-verbal memory tasks.

1.1.5.3. Non Verbal Memory

Pennington et al., (2003) reported deficits in relation to mentally age matched controls on several tasks that map onto the MTL, using a virtual version of the Morris water maze (MWM), and a selection of tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB). The virtual version of the MWM is adapted from the rodemt version of the task, whereby extramaze cues must be used to locate a hidden platform. Animals with dorsal or large hippocampal lesions usually present with a deficit on the MWM task; lesioned rats show increased latency to find the escape platform, and spend less time in the target quadrant of the maze when the platform is removed and they are allowed to search freely (Mumby, Astur, Weisend, & Sutherland, 1999). The virtual version of the task involves participants using a joystick, and using extramaze cues to navigate around a virtual room in order to find a hidden rug. On the virtual version of the task, the DS group spent significantly less time searching for the target in the

correct quadrant of the virtual environment, than the mentally age matched controls. In addition, Pennington et al., (2003) measured performance on the CANTAB pattern recognition memory test. On the latter test, children were presented with a series of 12 abstract patterns, for 3s each. On each recognition trial, children were presented with two patterns: one they had already seen and another new novel pattern, and were instructed to select the familiar pattern. DS participants were poorer in recognizing a previously presented pattern than their age matched controls. Pennington et al., (2003) also tested DS individuals on the CANTAB paired associate learning task, which requires learning an association between a visual stimulus, and its spatial location. This task is impaired in individuals who have hippocampal damage (Pennington et al, 2003). The CANTAB version of the task presented children with a series of boxes arranged in a circular pattern, and each of the boxes was removed in turn, to reveal a unique pattern underneath. A single pattern was then presented in the centre of the screen and the children were instructed to touch the box where that pattern had been shown during the presentation phase. DS participants performed significantly worse on the task than the age matched control group. In summary, the DS group demonstrated poorer performance on the hippocampal measures which were evaluated. The poor performance of DS individuals on spatial navigation, spatial recognition and object recognition tasks suggest aberrant function of the hippocampus and perirhinal cortex in DS.

Not all studies have successfully identified a problem with visuospatial memory in DS. Individuals with DS have been shown to demonstrate relatively preserved visuospatial memory when asked to recall where objects previously appeared within a display placed directly in front of them. For example, Wang and Bellugi (1994) found that DS participants performed at a similar level to mentally age matched typically developing individuals when carrying out the Corsi block tapping task, which requires subjects to observe the sequence of blocks "tapped" by a demonstrator, and then mimic the sequence back in the correct order. The task usually starts with a small number of blocks and gradually increases in length up to nine blocks, with the accuracy and length of the sequence remembered being recorded. Laws (2002) reported visuospatial memory in DS individuals which was better than that of controls, whom were matched for receptive vocabulary, on this task. Finally, in a study comparing visual object versus visuospatial memory, individuals with DS exhibited better recall for the previously seen spatial location of an object than for the form of the object, implicating abnormal activity of the perirhinal cortex (Vicari, Bellucci, & Carlesimo, 2005).

Additionally, a study by Edgin, Pennington, and Mervis (2010), failed to demonstrate a deficit in the ability of DS individuals to perform on a test of spatial navigation using the

computer – generated arena; the virtual version of the MWM. The authors pointed out that the task had problems with floor effects, and that scores were correlated with visuomotor task scores; perhaps the motor demands of the joystick required for the task are problematic for generating non-confounded data. It is also worth bearing in mind that the ethological validity of virtual environments to assess allocentric processing and memory in humans has been questioned (Taube et al., 2013). Whilst sitting in front of a computer screen, there is absence of physical movement and proprioceptive feedback.

An alternative study by Courbois et al. (2013) investigated wayfinding behavior of DS adults in a virtual town containing three target buildings and a number of visual landmarks, which has better face validity than the virtual MWM. Participants were trained on two different routes through the town. All chronologically age matched controls, 9/10 mentally age matched controls, and 7/10 participants with DS successfully learned the routes. However, participants with DS learned fewer landmarks when compared to the chronologically age matched control group. It should be considered, however, that although this may indicate a problem with spatial memory, this may result from the poor vocabulary displayed by individuals with DS. Edgin (2013) has also gone on to demonstrate specific problems with place learning without the use of a virtual environment. Using a task whereby individuals had to locate a hidden object within a rhomboid shaped room, children had to use either their own egocentric sense of direction to find the item, by entering the room from a familiar point or, after a period of disorientation (caused by spinning blindfolded on a chair) had to use geometric and landmark information to find the object. When relying on the directional information in reference to the child's own body in the egocentric condition, children with DS performed the task with accuracy, finding the target item in 92% of cases. However, following disorientation those individuals with DS were unable to use the spatial orientation of the environment to guide their searches and were incapable of combining geometric and landmark information in order to find the item. However, it is unclear whether the order of these tests were counterbalanced. These results further suggest that the ability to use some forms of spatial memory is impaired in DS, and again points towards dysfunctionality in the MTL.

A similar study by Lavenex et al. (2015) monitored participants who were asked to find three rewards hidden among 12 potentially rewarded locations distributed in a 4m x 4m arena, either in the presence of local cues (marked by a red cup), or in the absence of local cues which meant that participants had to find the rewards based on their relation to distal environmental cues. All age-matched controls found the rewards at above chance level both in the presence and the absence of the local cues. In contrast, all but one of the participants with DS exhibited a preference for the rewarded locations when

local cues were present, whereas only 50% of participants with DS chose the rewarded locations at above chance level in the absence of local cues. This further demonstrates that individuals with DS are impaired at using an allocentric representations to learn and remember spatial locations in a real-world environments. However, because the order of training on the local and distal cues was not counterbalanced, it also remains possible that prior training with the local cues blocked learning about the distal cues in DS individuals and the impairment may reflect an attention problem more than impaired processing of spatial information.

1.1.5.4. Visual stream processing

It could be proposed that the failure of DS participants to perform well on novelty and spatial recognition tasks is due to aberrant processing in the visual stream, due to a reduction in dendritic branching in the visual cortex (Becker, Armstrong and Chan, 1986). However, although only minimal behavioural studies have been carried out to interrogate the visual stream, basic visual perception tasks have shown that visual processing is intact in DS individuals. Brown et al., (2003) showed that 2–3year-olds with DS were unimpaired at visual tracking and integration compared to both mentally age matched and chronological age matched controls. In addition, Fidler, Hepburn and Rogers (2006) investigated the performance of 2 to 3-year-olds with DS on the Mullen Scale of Early Learning (MSEL) in comparison to mentally age matched controls; both typically developing, and those with other developmental disabilities. The MSEL is a standardised developmental test for children ages 3 to 60 months and consists of five subscales, one of which is visual reception. The visual reception element of the scale focuses on visual perceptual ability, and is used to assess visual discrimination and memory. Results demonstrated that children with DS showed no deficit on the visual perception aspect of the MSEL. The data suggest integrity in the visual stream processing, suggesting that memory dysfunction is less likely a result of aberrant inputs from the ventral cortex to the MTL, and is more likely a result of dysfunction in the hippocampus or other regions of the MTL, as described.

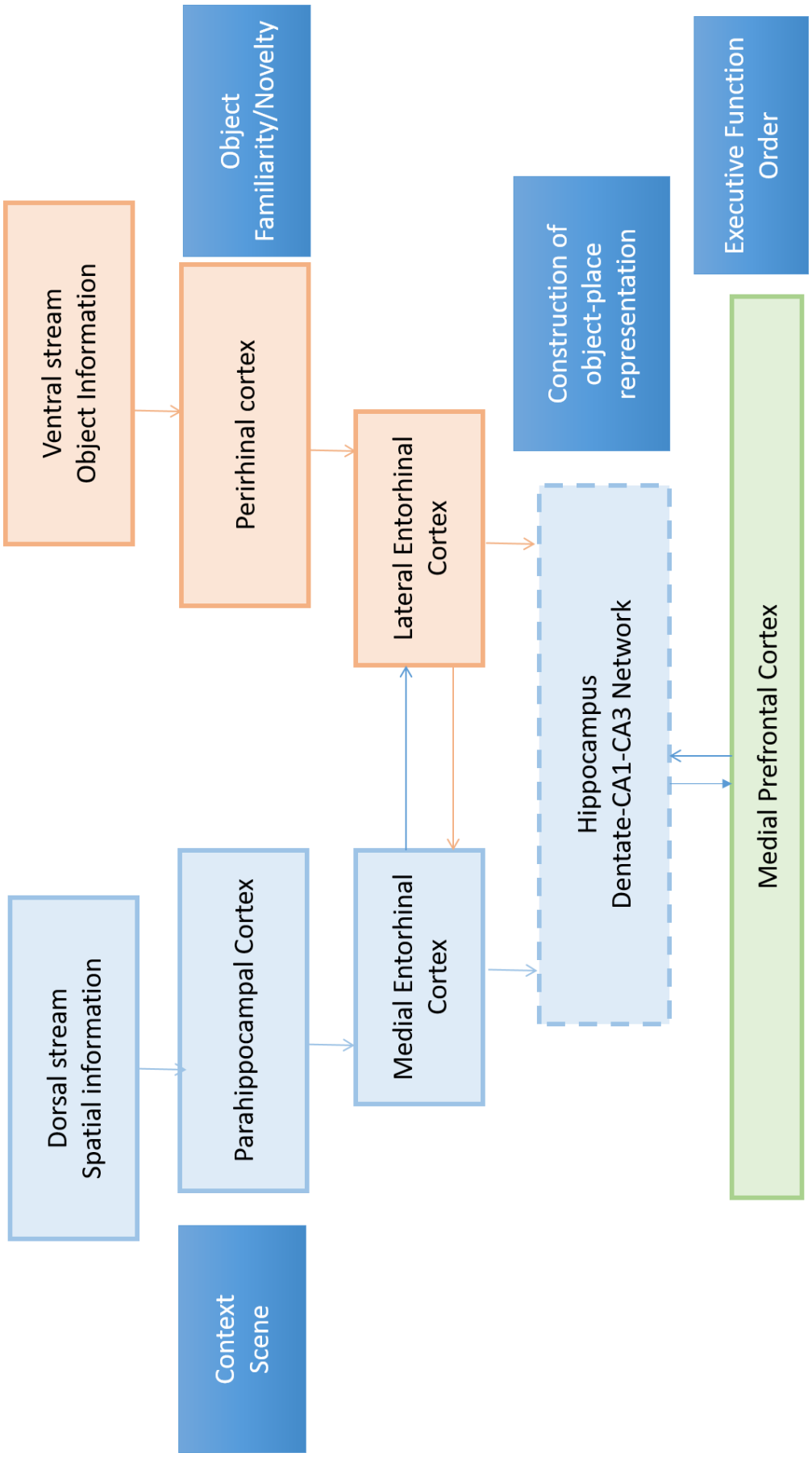


Figure 1.2- *Figure illustrating the processing streams of the MTL*

1.1.5.5. Prefrontal Cortex Function

The prefrontal cortex (PFC) is responsible for controlling a variety of functions which tend to be grouped under the term 'executive function'. Executive function relates to our ability to hold incoming information in our minds, and to plan tasks (Gilbert & Burgess, 2008). More specifically, these functions include working memory abilities, and the ability to inhibit actions for which a response tendency has been established. Executive function also refers to an ability to set-shift; to switch fluidly between response sets for different tasks (Miyake & Friedman, 2012). These abilities are important for performing everyday tasks, and to update incoming information, predict the consequences of actions and to perform in a socially acceptable manner; ultimately to facilitate behaviour in unfamiliar situations (Gilbert & Burgess, 2008). It has often been debated whether working memory, inhibition and set shifting are unitary or separable processes. Miyake et al. (2000) established a framework which deemed that the processes were correlated, but fractionated. Miyake carried out a study which examined the performance of 137 college students on a range of tasks which tapped into each element of executive function, as well as using a set of well-established tests of complex executive function, including the Wisconsin Card Sorting Task (WCST) and the Tower of Hanoi. It was confirmed that working memory, inhibition and set shifting are clearly separable. Moreover, Miyake et al. (2000) also suggested that the three functions contribute differentially to performance on complex executive tasks. For example the WCST was dependent on set-shifting ability, whereas performance on the Tower of Hanoi was related more to inhibition, suggestive of the unity of the different elements of executive function.

Numerous studies which discuss PFC lesions make reference to executive dysfunction. The idea of the component processes of executive function being separable entities is reinforced when examining lesions of the PFC in humans. It is generally accepted that there are three main frontal circuits (Alvarez & Emory, 2006). The dorsolateral frontal cortex has been linked to verbal fluency, set-shifting, and working memory (Alvarez & Emory, 2006). Lesions to the ventromedial circuit cause a lack of motivation, and finally, lesions to the orbitofrontal cortex cause a lack of inhibition and impulsivity (Alvarez & Emory, 2006).

Deficits in prefrontal function has been demonstrated in some studies of DS (Grieco, Pulsifer, Seligsohn, Skotko & Schwartz, 2015) specifically in working memory, attention and set-shifting, and in some studies, problems with inhibition. The fact that participants with DS show varying degrees of deficits on each of the elements of executive function supports Miyake's (2000) claim that the processes are indeed separable, or at least that the developmental trajectory for each of the processes is

separable. Much research has demonstrated difficulty with frontally dependent tasks. For example, Lanfranchi, Jerman, Dal Pont, Alberti and Vianello (2010) carried out a battery of tests of executive function in 15 DS adolescents and 15 mentally age matched controls. Within the battery were tasks which specifically probed set shifting, working memory and inhibition. The results revealed that the group of individuals with DS performed at a significantly lower level on each of the tasks for set-shifting, working memory and inhibition, suggesting pre-frontal functional abnormalities in DS.

Various other studies have carried out similar tasks which also demonstrate deficits in performance in DS participants on working memory and set shifting tasks (Brown et al., 2003; Rowe, Lavender, & Turk, 2006; Visu-Petra, Bellucci and Carlesimo., 2016; Zelazo, Burack, Benedetto, & Frye, 1996). Inhibition on the other hand is less consistently affected in DS. When Rowe et al. (2006), controlled for differences in verbal memory and psychomotor speed, only the measures of attention and set-shifting were significant. Furthermore, a study by, Edgin, et al. (2010) confirmed deficits in working memory and set-shifting in comparison to mentally age matched controls. However, on the Stroop task, only the working memory phase was affected in DS individuals, with no impairment on the element of the task that required only inhibition. These results point towards a consistent deficit in working memory and set shifting abilities in DS, and a less consistent deficit in inhibition. It must however also be noted that there may be age related changes in the prefrontal cognitive profile of DS participants. For example, in childhood some studies show aspects of preserved executive skills relative to individuals matched for mental age (Vicari et al., 2000; Pennington et al., 2003; Lanfranchi et al., 2010; Costanzo et al., 2013), whereas other studies demonstrate impairment in aspects of executive function for individuals with DS, (Trezise et al., 2008; Costanzo et al., 2013); longitudinal studies would allow for more clarity regarding this.

1.2. Modelling DS in rodents

In order to understand the aetiology of the disease, and to attempt to understand how the pathological elements of the disorder produce the resulting phenotypes, the development of transgenic mice has been imperative. Human chromosome 21 (Hsa21) expresses 233 protein-coding and 299 non-coding genes, together with 29 microRNAs (Siew, Tan, Babaei, Cheah, & Ling, 2013). In the mouse however, there is no equivalent of Hsa21; the homologous genes are split over three orthologous regions on mouse chromosomes (Mmus) 16 (around 145 genes), 17 (19 genes) and 10 (41 genes) as shown in figure 1.3. The fact that Hsa21 genes are split across three different Mmus means the modelling of trisomy 21 is difficult, because the equivalent mouse chromosomes have syntenies with other human chromosomes. For example trisomy for the entirety of chromosome 16 would result in trisomy for genes not syntenic to Hsa21, and a lack of genes syntenic to Hsa21 which are orthologous to Mmu17 and 10. Nevertheless, the general conservation of the Hsa21 genes in mice means that aspects of the DS phenotype can be modelled in mice.

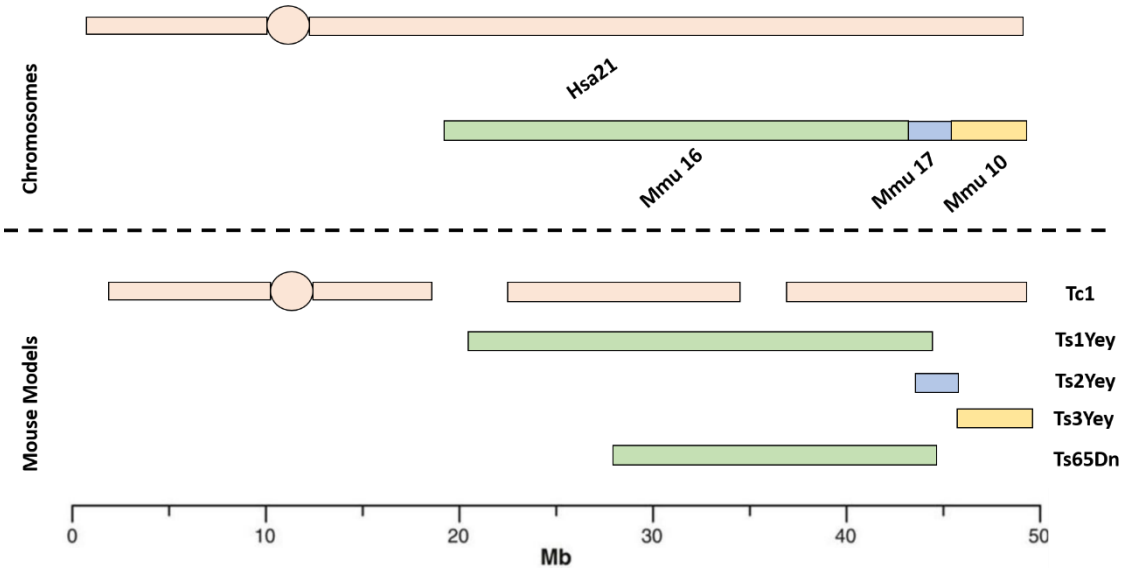


Figure 1.3- *Schematic diagram illustrating the genetic makeup of the different mouse models of DS. Adapted from Ruparelia et al., (2013).*

1.2.1. Mouse models of DS

Various genetic models of DS have been created, the majority of which are segmental trisomies which contain varying degrees of trisomy, for numerous chromosomal regions syntenic with Hsa21. A summary of the models can be found in table 1.1. The mouse models will be described below in their chronological order of publication, with genetic details and basic phenotypes being described first, before a more in depth discussion of synaptic plasticity changes in the models, and finally, the cognitive phenotypes and how they differ between models.

1.2.1.1. Ts16 mouse model

The first mouse considered to model DS was the Ts16 model, which was created by mating mice heterozygous for two Robertsonian translocation chromosomes containing Mmu16, to WT mice (Miyabara, Gropp, & Winking, 1982). Approximately 15-40% of the resultant offspring are trisomic for the shared Mmu16. Unfortunately, Ts16 mice are not viable, as they die shortly before birth with oedema, so cannot be used for any cognitive assessments. However, they are a reasonably useful model for developmental studies. Ts16 embyros have been shown to exhibit the same sorts of craniofacial dysmorphia, and congenital heart defects that are seen in DS individuals (Gearhart et al., 1986). Studies have also shown growth retardation, in that they exhibit both a small body and brain size when compared with control embryos (Lacey-Casem & Oster-Granite, 1994). Additionally, these animals also show a decrease in basal forebrain cholinergic neurones, and abnormal cell migration (Davisson, 2005). Considering that Mmu16 shares many genes with Hsa21, abnormalities in Ts16 brain development may parallel abnormalities in trisomy 21. However, there is a caveat in that defects which are seen may be a result of trisomy of Mmu16 genes which are not syntenic with Hsa21.

Mouse Model	Trisomic Regions
Ts16	Trisomic for entire Mmu16
Ts65Dn	Trisomic for the distal end of Mmu16 from Mir155 to zfp295, ~100 genes. Also trisomic for around 5-6MB of the proximal end of Mmu17
Ts1Cje	Trisomic for a small region of Mmu16, plus a monosomy for 7 genes on Mmu12.
Ts1Rhr	Smaller trisomic segment of Mmu16 that corresponds closely to the DSCR
Ts1Yah	Carry a 0.59-Mb duplication between *Abcg1* and *U2af1* in the Hsa21 syntenic region on Mmu17
Ts1Yey	Duplication of the segments of Mmu16 which are orthologous to Hsa21
Ts2Yey	Duplication of the segments off Mmu10 which are orthologous to Hsa21
Ts3Yey	Duplication for the segment of Mmu17 which is homologous to Hsa21
TTS (Triple Trisomic Mouse)	Trisomic for the Hsa21 syntenic regions of all three orthologous mouse chromosomes; 16, 17 and 10
Tc1	Contains a freely segregating and almost complete copy of Hsa21

***Table 1.1**- Table listing available mouse models of trisomy-21 (DS)*

1.2.1.2. Ts65Dn mouse model

The Ts65Dn model was the first segmental trisomy model to be created, and the trisomy is present as a freely segregating chromosome (Davisson et al., 1993). It is trisomic for only the distal end of Mmu16, as opposed to the whole of Mmu16, and this accounts for approximately 56.5% of Hsa21 (Li et al., 2007). The Ts65Dn is trisomic for the region of Mmu16 from Mir155 to zfp295, which is approximately 100 genes (Belichenko, Kleshchevnikov, Salehi, Reeves & Mobley., 2015). In addition, the model is also trisomic for around 5-6MB of the proximal end of Mmu17, however these genes are not orthologous to Hsa21 (Davisson et al., 1993). The Ts65Dn recapitulates many aspects of human DS, including delayed sensorimotor milestones, cholinergic system deficits, reduced cerebellar volume and craniofacial abnormalities. Despite the presence of a triplication of 60 centromeric genes on Mmu17 that are not present on Hsa21 (Duchon et al., 2011), the Ts65Dn remains the most commonly used, and thus best characterized mouse model of DS.

The Ts65Dn has made it possible to explore behavioural, physiological and neurobiological aspects of DS. Developmental phenotypes are evidenced by a significant reduction in size at weaning, along with muscular trembling and craniofacial differences (Davisson et al., 1993). They replicate many DS-like abnormalities, including a small mandible and other craniofacial phenotypes (Richtsmeier, Baxter, & Reeves, 2000). Ts65Dn mice also show alterations in the structure and function of dendritic spines in cortical and hippocampal neurones (Belichenko et al., 2004), and a lack of hippocampal long term potentiation (a model of the putative synaptic basis of memory; LTP) (Kleschevnikov et al., 2004). However, many DS phenotypes are not present, including cardiac defects, and skeletal abnormalities; in addition, no incidence of leukemia has been reported in Ts65Dn mice, and they do not show early onset of Alzheimer's disease (Reeves et al., 1995). Importantly though, when characterising the behavioural phenotypes in the Ts65Dn mouse, some deficits are consistent with DS. Ts65Dn mice for example show critical changes in their behaviour, including deficits on novel object recognition tasks, object location tasks and spatial navigation tasks, which will be discussed in more detail later in this chapter.

1.2.1.3. Ts1Cje mouse model

The Ts1Cje model is a segmental trisomy which results in trisomy for approximately 75% of the genes which are triplicated in the Ts65Dn, together with a monosomy for 7 genes on Mmu12 (Duchon et al., 2011). The model was created fortuitously during an experiment which aimed to target SOD1. The model has a less severe phenotype than that of the Ts65Dn mouse. It shows some learning and memory deficits and a less exaggerated craniofacial dysmorphology (Davisson, 2005). For example, the Ts1Cje mouse has been demonstrated to exhibit learning deficits in the MWM, however unlike the Ts65Dn mouse, they are able to perform the cued version of the task, and the deficit they show on the hidden platform version of the task is also less pronounced. Additionally, there is no degeneration of the basal forebrain cholinergic neurones as seen in the Ts65Dn mouse (Sago et al., 2000). In terms of the craniofacial abnormalities, the dysmorphology is generally similar to the Ts65Dn, but there is no broadening of the cranium (Richtsmeier et al., 2002). Although the model seems less accurate in recapitulating human DS, they are useful in that they can be used to study the effects of trisomy for a particular subset of genes which are triplicated in Ts65Dn, thus narrowing down the gene segments which when in triplicate, are sufficient to cause specific phenotypes.

1.2.1.4. Ts1Rhr and Ms1Rhr mouse models

The Dp(16Cbr1-ORF9)1Rhr (Ts1Rhr) mouse is trisomic for just the subset of mouse genes triplicated in the Ts65Dn and Ts1Cje which correspond to the Down Syndrome Critical Region (DSCR) (Davisson, 2005). The DSCR is a chromosome 21 segment which was purported to contain genes responsible for many of the features of DS, including the craniofacial features, a presumption which dominated the DS field (Olson, Richtsmeier, Leszl & Reeves, 2004). However the true impact of the DSCR had not been definitively tested until the creation of the Ts1Rhr and Ms1Rhr mice (Olson, Richtsmeier, Leszl & Reeves, 2004). The advantage of the Ts1Rhr is that it has been used to determine whether trisomy for the DSCR is sufficient to produce the phenotypes seen in Ts65Dn and DS. Additionally, the Ms1Rhr can be crossed with the Ts65Dn to 'remove' trisomy of the DSCR in order to determine whether the genes on the DSCR are necessary for DS phenotypes. Analysis of these models has demonstrated that trisomy for the 33 genes on the DSCR alone is insufficient and generally unnecessary to cause many of the consistent DS phenotypes, including altered stature, small mandible and anomalies of the craniofacial skeleton, which provided significant evidence against the concept of the DSCR (Olson et al., 2007). Furthermore, reducing trisomy of these 33

genes to disomy in the Ts65Dn mouse did not eliminate the phenotype (Olson et al., 2007). Ts1Rhr mice exhibited a larger overall size and craniofacial alterations (Deitz & Roper, 2011). Additional studies of Ts1Rhr mice and their euploid littermate controls have also shown differences in brain size; Ts1Rhr have a relatively smaller brain overall, the cerebellar proportion of overall volume is unaltered in the Ts1Rhr (Aldridge, Reeves, Olson, & Richtsmeier, 2007). As a result of the generation of the Ts1Rhr and Ms1Rhr, Olson et al., (2007) favour the idea that this small number of genes can make a critical contribution to DS, but that their effect is highly dependent on the altered dosage of other genes on chromosome 21. In order to further investigate the impact of trisomy of genes on chromosome 21, it would be interesting to increase the size of the trisomic regions, however the number of genetic permutations would not make this a simple task.

1.2.1.5. Ts1Yah mouse model

The Ts1Yah is trisomic for the region between *Abcg1–U2af1* on the telomeric region of Hsa21, which maps to Mmu17 and Mmu10 (Pereira, Magnol, Sahún, et al., 2009). In addition, the corresponding monosomic model, the Ms2Yah was also created. The aim of this model was to interrogate regions outside of the DSCR, such as the telomeric region of Hsa21 in order to establish whether they may contribute to the DS phenotype. Behavioural and neurological analysis of the Ts1Yah mice has shown a clear but complex cognitive phenotype that combines altered short-term spatial memory and short-term recognition memory in the NOR (Pereira, Magnol, Sahún, et al., 2009). However, the specific trisomy in the Ts1Yah improved the performance in spatial learning in the spatial version of the MWM; an unexpected result considering that other mouse models of DS demonstrate a deficit on this task (Pereira et al., 2009). The model mimics DS patients for their reduced attention and their alterations in processes involved in some forms of explicit memory that require the integrity of hippocampal functions. The model however is not widely used. The fact that the model has some similarities but also some clear differences to other models of trisomy 21 demonstrates that trisomy of specific regions can both decrease certain cognitive functions and improve others. Thus the contribution of the trisomy of different regions of Hsa21 to different DS phenotypes needs evaluating further.

1.2.1.6. The Ts1Yey; Ts2Yey; Ts3Yey and the triple trisomy mouse model

Three complementary segmental trisomy models were created using Cre/loxP engineering technology. The Ts1Yey is a segmental trisomy model which has a duplication of the whole Hsa21 syntenic region of Mmu16. The Ts2Yey has a duplication of the segments off Mmu10 which are orthologous to Hsa21, and finally, the Ts3Yey has a duplication for the segment of Mmu17 which is homologous to Hsa21. The most complete mouse model of DS was then generated by Eugene Yu in 2010 (Yu et al., 2010), by crossing together the three segmental trisomy models to generate the triple trisomy mouse. Two of the mutants were first bred together to generate compound mutants, which were then crossed with the mutant carrying the third duplication. The triple trisomic mouse is produced at normal Mendelian ratios, and is trisomic for the Hsa21 syntenic regions of all three orthologous mouse chromosomes; 16, 17 and 10 (Yu et al., 2010).

The availability of mice that are trisomic for varied subsets of the Hsa-21 orthologous genes provides a useful tool to identify which genes contribute most to cognitive phenotypes of Down syndrome. Looking at trisomy of small subsets of genes and their resultant phenotypes will eventually allow for a description of genes which are necessary and/or sufficient to produce a particular phenotype. This is more efficient than attempts at pinning down single genes which may cause complex phenotypes in DS. Aspects of cognitive function that map to different brain regions have been tested in these mouse models and some deficits have parallels with cognitive phenotypes of DS. A common finding in mouse models is the presence of impaired synaptic plasticity and learning and memory. These features will be reviewed in the next section.

1.2.2. LTP and LTD changes

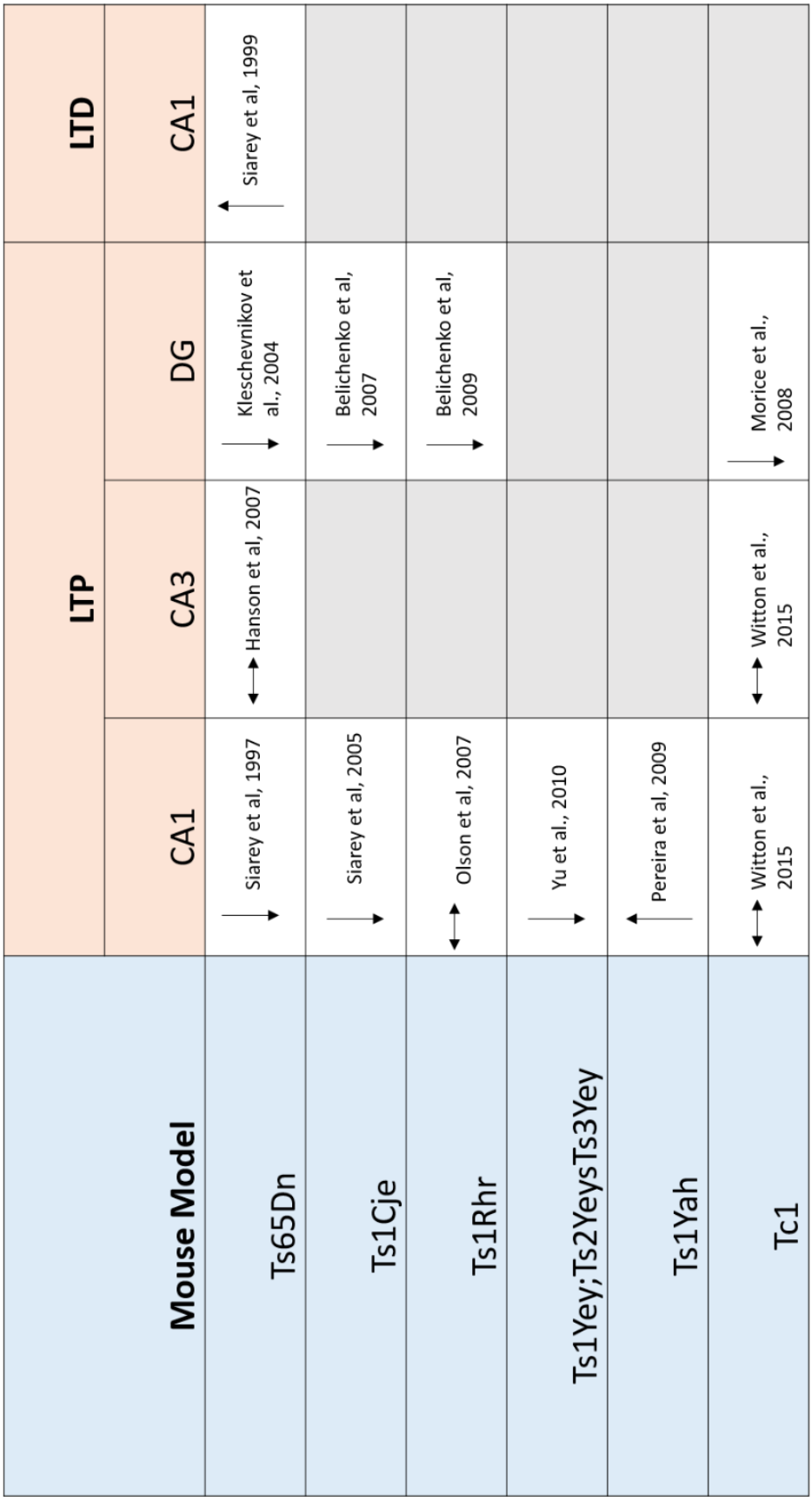
Long term synaptic plasticity, a putative physiological mechanism for the synaptic basis of learning and memory, can be measured as changes in synaptic efficacy and excitability between presynaptic and postsynaptic neurons. Enhancement in potentiation that persists for long periods of time is called long-term potentiation (LTP). Conversely, a persistent decrease in potentiation, or depression, is called long-term depression (LTD). It has been demonstrated that many DS models show reduced hippocampal LTP (see Table 1.2).

Studies have explored synaptic plasticity in the different hippocampal regions; the CA1, CA3 and dentate gyrus (DG). In the Ts65Dn mouse, induction of LTP and LTD was investigated by recording field excitatory postsynaptic potentials (EPSPs) from the CA1 region of hippocampal slices, and LTP and LTD evoked sequentially (Siarey et al., 1999; Siarey, Stoll, Rapoport, & Galdzicki, 1997). It was found that LTP decreased whereas LTD increased significantly in Ts65Dn compared with control hippocampus. Despite normal basal synaptic transmission in the dentate gyrus of Ts65Dn, there was also severe impairment of LTP in the DG (Kleschevnikov et al., 2004). After suppressing inhibition in the dentate gyrus with picrotoxin, a $GABA_A$ receptor antagonist, induction of LTP was restored, suggesting that enhanced inhibitory synaptic transmission is the primary cause of the failure to induce LTP in the dentate gyrus of Ts65Dn mice. Similar results have also been observed in the CA1 region (Siarey et al., 1997, 1999). One of the possible mechanisms which could be responsible for altered GABAergic transmission in the Ts65Dn is the overexpression of Grik1, the gene encoding the GluR5 subunit of the kainate receptors (Kleschevnikov et al., 2004). Grik1 is present in three copies in Ts65Dn and GluR5-containing receptors are present in GABAergic neurons within the hippocampus (Paternain, Herrera, Nieto, & Lerma, 2000). The selective activation of GluR5 receptors has been show to increase inhibition in the CA1 region of the hippocampus (Cossart, Esclapez, Hirsch, Bernard, & Ben-Ari, 1998), and it would thereby follow that an overexpression of GluR5 in the DG of Ts65Dn mice may also lead to increased inhibition.

Similarly, baseline synaptic efficiency and synaptic plasticity were examined in the DG in hippocampal slices of triple trisomic (TTS) and WT mice (Belichenko et al., 2015). There was no difference in the baseline efficiency of excitatory neurotransmission in TTS mice when compared to WT slices, however, where tetanizations readily induced LTP in WT mice, this LTP induction was not observed in TTS slices. Again, levels of LTP were measured following the suppression of the $GABA_A$ receptor by administration of

picrotoxin, and this suppression of inhibition returned LTP induction to a comparable level to WT slices in TTS slices.

A reduction in LTP has also been observed in the Ts1Cje mouse (Siarey, Villar, Epstein, & Galdzicki, 2005) and in the Ts1Yey, Ts2Yey and Ts3Yey (Yu et al., 2010). In contrast, the Ts1Yah showed increased LTP in the CA1 region of the hippocampus (Pereira, et al., 2009), and Ts1Rhr LTP appears to remain unaltered in comparison to control mice (Olson et al., 2007). Ultimately though, the impaired induction of LTP which is seen in a number of mouse models of DS seem to point towards enhanced inhibitory mechanisms in the hippocampus due to unbalanced excitatory and inhibitory neurotransmission. It is thought that reduced activation of NMDA receptors could hinder LTP in trisomic mice, and that increased LTD is mediated by exaggerated NMDA receptor dependent mechanisms (Scott-McKean & Costa, 2011). Additionally, overinhibition in the hippocampus of Ts65Dn mice has been shown to be dependent on $GABA_A$ receptors; a $GABA_A$ antagonist, picrotoxin has been shown to rescue the reduced LTP in these mice (Costa & Grybko, 2005). However, it must be noted that the Ts65Dn mouse is trisomic for genes which are not syntenic to chromosome 21, and so this increased inhibition may in fact be as a result of trisomy of a gene which is unrelated to DS. Additionally, the G-protein-activated inwardly rectifying potassium channel 2 (Girk2) gene is overexpressed in DS individuals. Girk channels generate a $GABA_B$ receptor-dependent slow inhibitory postsynaptic potential in hippocampal neurons (Lüscher, Jan, Stoffel, Malenka & Nicoll, 1997), and it has been proposed that an increase in Girk2 gene expression may produce overinhibition in hippocampal neurons and contribute to LTP failure in the trisomic condition (Best, Siarey & Galdzicki, 2007).



Mouse Model	LTP			LTD
	CA1	CA3	DG	CA1
Ts65Dn	Siarey et al, 1997 →	↔ Hanson et al, 2007	→ Kleschevnikov et al., 2004	← Siarey et al, 1999
Ts1Cje	Siarey et al, 2005 →		→ Belichenko et al, 2007	
Ts1Rhr	↔ Olson et al, 2007		→ Belichenko et al, 2009	
Ts1Yey;Ts2YeysTs3Yey	Yu et al., 2010 →			
Ts1Yah	← Pereira et al, 2009			
Tc1	↔ Witton et al., 2015	↔ Witton et al., 2015	→ Morice et al., 2008	

Table 1.2 *- Table listing LTP deficits in different mouse models of trisomy-21. (Upward pointing arrow = increased, downward pointing arrow = decreased, horizontal arrow = unaltered).*

1.2.3. Cognitive Phenotypes

The LTP deficits are coincident with behavioural deficits in learning and memory tasks. The next section will describe the behavioural phenotypes of the different mouse models in more detail (see Table 1.3). Behavioural tests can be used to assess defects in the function of specific brain regions. Behavioural tests may therefore help to define the brain regions (or systems) that are especially affected models of DS (several of which will be described in more detail subsequently). For example, in object recognition memory, the perirhinal cortex is responsible for encoding object information, but the hippocampus for memory of the spatial context in which objects are presented (Barker & Warburton 2011). The different anatomical structures and circuits involved in these behavioural tasks allows for the investigation of their sensitivity toward trisomy of Hsa-21 genes and how these brain regions may therefore be affected in DS.

1.2.3.1. Spontaneous object recognition memory

Novel object recognition utilises rodent's innate preference for exploring novelty over familiarity. When rodents are presented with two copies of object A in an open arena, when subsequently presented with a copy of object A together with a copy of object B, rats will typically spend longer exploring the novel (Ennaceur & Delacour, 1988). This simple protocol has been utilised for a vast array of experiments which test for recognition memory. Numerous experiments have also demonstrated that lesions to the perirhinal cortex impair recognition memory in rats when they are tested on the simple spontaneous object recognition paradigm (Aggleton, Keen, Warburton, & Bussey, 1997; Ennaceur & Aggleton, 1997; Ennaceur, Neave, & Aggleton, 1996). Many studies have reported no effect of hippocampal lesions on behavioural paradigms that test recognition memory (Aggleton, Hunt, & Rawlins, 1986; Albasser, Amin, Lin, Iordanova, & Aggleton, 2012; Barker & Warburton, 2011; Kesner, Bolland, & Dakis, 1993; Langston & Wood, 2010; Mumby, Pinel, Kornecook, Shen, & Redila, 1995; Steele & Rawlins, 1993). However, there are also reports of hippocampal lesion induced deficits on object novelty tasks in rodents (Clark, Zola, & Squire, 2000; Cohen et al., 2013; Yi, Park, Kim, Kim, & Ryu, 2016).

Spontaneous preference tasks are very versatile and have been adapted to measure memory for object location (Ennaceur, Neave, & Aggleton, 1997) and object-in-place information (Dix & Aggleton, 1999). Rodents can remember if a familiar object has been moved to new position. The object in place test requires that mice are presented with items in the sample phase, which remain in a consistent spatial location, and during the test phase, one of the items is moved to a novel location. Rodents will typically spend

longer exploring the item in the novel location than they spend exploring the item in the familiar location. The hippocampus is central to processing spatial aspects of memory; this has been demonstrated across many experimental paradigms including electrophysiological recording studies and lesion studies (Albasser et al., 2013; Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe & Dostrovsky, 1971; Warburton & Brown, 2010).

Ts65Dn mice have been shown to display impaired novel object recognition in various versions of the task (Fernandez et al., 2007). Mice were presented with the objects for 15 minutes during a sample phase, and tested 24 hours later in a 15 minute test phase in order to evaluate long term object recognition memory. Ts65Dn mice exhibited significantly impaired novel object recognition. Ts1Rhr mice show a similar pattern of object recognition memory deficits as the Ts65Dn (Belichenko et al., 2009). Mice were placed in a cage with two novel objects and after 1 h or 24 h delay were reintroduced into the box which now contained one novel and one familiar object. Ts1Rhr mice were impaired at both delays. Interestingly, the Ts1Cje mice are able to discriminate between novel and familiar objects (Fernandez et al., 2007). The ability of Ts1Cje mice to detect object novelty, may point to differences in pathology in the DS mouse models. This is intriguing considering the genes which are triplicated in each of the models. Ts1Cje mice are trisomic for 80% of the genes in Ts65Dn mice and all genes in Ts1Rhr mice, yet Ts1Cje mice show no deficit in the novel object recognition task (NORT) (Pereira et al, 2009), thus the genetic make-up does not readily explain the phenotypic differences.

The role of the hippocampus in recognition memory is controversial; it is thought that the role of the hippocampus is necessary for the learning of spatial information, thus, an intact hippocampus is important for the object-in-place task, but not for NORT (Barker & Warburton, 2007). Interestingly, Ts65Dn mice perform normally in the object-in-place task (Fernandez & Garner, 2008). Furthermore, an earlier report using 10- to 12-month-old Ts65Dn mice found them to be normal in the object-in-place task and NOR (Hyde & Crnic, 2002). Thus, evidence suggests that the perirhinal cortex might make important contributions to NOR deficits in the Ts65Dn mouse model, and in other mouse models of DS. Spontaneous novel object recognition was also used to examine long-term memory in TTS and WT mice (Belichenko et al., 2015). During the acquisition phase and the test phase, TTS mice and their WT littermate controls spent equal amounts of total time exploring objects suggesting no difference between the groups, however, the discrimination ratios were significantly diminished in the TTS mice in comparison to the controls, pointing towards a deficit in their ability to detect the novel object following a 24 hour interval. Experiments interrogating spatial recognition memory abilities in the Ts1Rhr and Ts1Cje mice remain to be carried out, and further investigation into the

spatial learning abilities of these models could potentially help to elucidate the genes which, in trisomy, cause a deficit to be seen in this task.

Smith, Kesner, and Korenberg (2014) hypothesized that in the Ts65Dn mouse, the specific role of the DG is disturbed in its support of contextual and spatial information. Smith, Kesner and Korenberg (2014) developed a novel series of spontaneous exploratory tasks that emphasize the importance of recognizing spatial and contextual cues, and that involve DG function. The test paradigm used short delays (5 min) within a red box that prevented the use of context and a clear box with stimuli that could be encoded as context. The Ts65Dn mice demonstrated intact object recognition in the red box, but an inability to discriminate between familiar and novel when in the clear box. This suggests that Ts65Dn mice are able to detect novel objects following a five minute delay, however they are impaired when there was an important contribution of context. These results are indicative of spared object recognition memory independent of contextual information. In the clear box however, there is a potential for spatial interference between local object cues and the context cues. One of the functions of the DG is to decrease spatial interference (Kesner, 2013). Thus, the difficulty with object recognition in the presence of spatial interference may point towards aberrant DG function in the Ts65Dn. When the delay period between sample phase and test phase was increased to 24 hours, Ts65Dn were unable to detect the novel object in both the red and clear box conditions, indicating a long term object recognition memory deficit, independent of context information. Additionally, when tested on the object in place recognition task, Ts65Dn mice were impaired at 5 minute delays in both the red and clear box, suggesting that the representation of spatial location information is also impaired. Double dissociation studies have confirmed that animals with perirhinal lesions are impaired on object recognition but not spatial memory tasks, and animals with hippocampal lesions are impaired on spatial but not object recognition memory tasks (Kealy & Commins, 2011). Thus, it appears that the Ts65Dn mice have a dysfunction of the DG, which causes problems with spatial recognition abilities, and also affects the processing of object recognition memory when there is interference of contextual information. It would be interesting to carry this same experiment out in the partial trisomy models, in order to narrow down the combination of trisomic genes responsible for this phenotype.

Behavioural Task	Mouse Model					
	Ts65Dn	Ts1Cje	Ts1Rhr	Ts1Yey;Ts2Yey; Ts3Yey	Ts1Yah	Tc1
Novel Object Recognition	**Impaired** (Fernandez et al., 2007; Kleschevnikov et al., 2012; Torre et al., 2014) **Normal** (Hyde and Crnic, 2002)	**Normal** (Fernandez and Garner, 2007)	**Impaired** (Belichenko et al., 2009)		**Impaired** (Pereira et al., 2009)	**Impaired STM Normal LTM** (O'Doherty et al., 2005; Morice et al., 2008)
Object-in-Place	**Normal** (Fernandez and Garner, 2008; Hyde and Crnic, 2002)	**Normal** (Fernandez and Garner, 2007)				
Morris Water Maze (Cued)	**Impaired** (Reeves et al., 1995; Netzer et al., 2010)	**Normal** (Sago et al., 1998)	**Normal** (Olson et al., 2007)	**Normal** (Yu et al., 2010)	**Normal** (Pereira et al., 2009)	**Normal** (Morice et al., 2008)
Morris Water Maze (Hidden Platform)	**Impaired** (Reeves et al., 1995)	**Impaired** (Sago et al., 2008)	**Normal** (Olson et al., 2007)	**Impaired** (Yu et al., 2010)	**Normal** (Pereira et al., 2009)	**Normal** (Morice et al., 2008)
Contextual Fear Conditioning	**Impaired** (Costa et al., 2008; Salehu et al., 2009)			**Normal** (Yu et al., 2010)		

Table 1.3 *- Table listing behavioural phenotypes of different mouse models of trisomy-21*

1.2.3.2. Spatial recognition

The MWM assesses spatial navigation abilities of rodents. The MWM is carried out in a circular tank of water, made opaque by adding milk powder. A transparent platform is submerged approximately 2 cm below the surface of the water. In the cued version of the task, mice learn to find the platform with a visible cue such as a flag; mice learn a sequence of movements in response to a proximal cue. In the hidden platform version of the task, the platform is not associated with a visible cue, the mice must swim around until they locate the platform. The position of the platform is kept constant, but the starting position of the mouse is altered with each trial (Morris, 1981). Spatial mapping and the use of extra-maze cues must be learned to find the platform. Performance on the task is assessed by measuring the latency time to find the platform, path length the type of trajectory used to arrive at the platform (Morris, 1981).

In a study by Escorihuela et al., (1995) the performance of Ts65Dn mice on the Morris water maze task was examined, and it was found that place learning acquisition on the hidden platform task was impaired. Ts65Dn mice show an improvement in escape latencies across trials, however, the escape latencies are impaired in comparison to control mice (Escorihuela et al., 1995). Trisomic mice showed increased distance travelled, particularly in the peripheral ring of the maze. Thus, when only the inner portions of the quadrants were analysed, it became clear that the Ts65Dn showed poor discrimination in the inner parts of the quadrant. This indicated that the trisomic mice were likely using a search strategy whereby they swam in concentric circles until they happened upon the platform, as opposed to using the more effective strategy of using extra maze cues to learn and remember the location of the platform (Escorihuela et al., 1995). By comparison, when performing the cued version of the task, which relies on mice forming an association between a white flag and the presence of the platform, and the use of spatial information is not necessary, Ts65Dn showed no deficit in comparison to control mice. Holtzman et al. (1996) demonstrated the same pattern of reduced latency to reach the platform in the hidden platform version of the task. Sago et al. (2000) also demonstrated that whilst WT mice demonstrated improved latency to find the platform over the course of trials, Ts65Dn mice did not.

Most reports indicate that trisomic mice performed at a comparable level to control mice in the cued version of the task (Escorihuela et al., 1998; Rueda, Florez, & Martinez-Cue, 2008; Sago et al., 2000). However, when required to use extra-maze cues, the Ts65Dn, Ts1Cje and Ts1Yey mice showed impaired learning and memory in the hidden-platform task (Reeves et al., 1995; Sago et al., 1998). This is in corroboration with human studies of DS individuals. Ts1Rhr mice do not show impairment in the MWM in either cued or

hidden-platform tasks (Olson et al., 2007). Triple trisomic mice demonstrated impaired performance in the MWM (Yu et al., 2010), but trisomy for only the Mmu10 or Mmu17 segments does not result in impairment in this task. In fact, Ts1Yah mice performed better in the hidden platform task than their euploid littermates (Pereira et al., 2009; Yu et al., 2010). The hidden platform version of the task is corroborated with hippocampal lesions; rats with hippocampal lesions show no sparing of spatial memory, spending less time in the target quadrant, and showing a reduced latency to reach the platform location (Clark, Broadbent, & Squire, 2005). This is suggestive of hippocampal deficits in Ts65Dn mice which is translated to behavioural deficits, which are reflective of human DS.

1.2.3.3. Fear Conditioning

In classical fear conditioning, a conditioned stimulus (CS) such as a tone or context is paired with an unconditioned stimulus (US), such as an aversive electric shock that naturally generates a freezing response in rodents. When the CS is subsequently presented in the absence of the US, it can still induce a freezing due to the association of the stimuli (Carew, Walters, & Kandel, 1981). In contextual fear conditioning, a mouse learns to associate a chamber, or specific context, with being shocked; whereas in cued fear conditioning, a mouse associates a sound with being shocked. Contextual fear conditioning is a hippocampal dependent task, while cued fear conditioning is not (Jarrard, 1993; Phillips & Le Doux, 1992).

Ts1Yey, Ts65Dn and triple trisomy mice all show a reduced freezing response when reintroduced to a conditioned context compared with WT littermates (Hyde & Crnic, 2002; Salehi et al., 2009; Yu et al., 2010). This suggests that the function of pairing contextual CS to the US is impaired, which points towards aberrant hippocampal function in these models. The Mmu10 and Mmu17 segmental trisomies, Ts2Yey and Ts3Yey, each show a normal response (Yu et al., 2010). Ts65Dn mice show a normal freezing response to cued acoustic conditioning (Salehi et al., 2009). This suggests that pairing acoustic CS to the US is intact in Ts65Dn mice, which is indicative of intact amygdala function.

1.2.4. What can we learn about DS from mouse models?

By increasing our understanding of the relationship between genetic changes and behaviour using mouse models, we can build stronger theories concerning the nature of the cognitive changes in DS individuals and the neural substrates that underpin them. Ultimately, this may lead to specific drug targets to counter genetic changes. The mouse models vary quite significantly in their genetic make-ups. The Ts1Cje mouse model for example, is trisomic for a much smaller proportion of genes that are orthologous to Hsa-21 than the Ts65Dn, and they show a much milder cognitive phenotype, in that they demonstrate normal recognition memory and a less severe deficit on spatial recognition memory (Fernandez and Garner, 2007). Evidence from the Ts1Rhr, however, would suggest that the presentation of particular phenotypes is less to do with the number of trisomic genes, and more to do with the combination of genes that are triplicated. Ts1Rhr mice for example have impaired object recognition, but normal spatial working memory (Belichenko et al, 2009; Olson et al, 2007) despite being trisomic for only approximately half of the genes which are trisomic in the Ts1Cje model. The task of deciphering which permutations of gene triplication are responsible for particular phenotypes is a daunting prospect, but the development of a number of segmental trisomy models will help to elucidate the mechanisms by which trisomy of genes on human chromosome 21 may result in specific phenotypes. It could be argued that in the case of DS, the strength of mouse models lies not with their comparison to the human condition. Rather, the strength of mouse models of trisomy-21 lies with their ability to effectively map brain regions and cognitive changes affected by trisomy of specific genes.

Various mouse models have been developed in order to further study DS, however due to the fact that Hsa21 genes are split across three different Mmus, the modelling of trisomy 21 is difficult, because the equivalent mouse chromosomes have syntenies with other human chromosomes. The development of the Tc1 mouse (O'Doherty et al, 2005) has the potential to overcome some of these difficulties, as the mouse contains a freely segregating copy of the human chromosome 21. Using the transchromosomic approach more closely mirrors a full human trisomy, and does not interrupt endogenous mouse sequences. A major aim of this thesis was to characterise the cognitive phenotype in the Tc1 mouse model in order to provide insight into the learning and memory systems disrupted by the mutation. The Tc1 model will be described in more detail below.

1.2.5. The Tc1 mouse

The Tc1 mouse model of DS was developed by (O'Doherty et al., 2005), and is a transchromosomic mouse strain which carries a freely segregating and almost complete human chromosome 21; Tc1 mice are functionally trisomic for around 75% of human genes due to genomic rearrangements that occurred whilst generating the mouse (Gribble et al., 2013). There is also a significant degree of mosaicism, the percentage of which varies from mouse to mouse, and also within different tissues within a particular mouse (Das & Reeves, 2011). Mosaicism however is also seen in human DS, potentially resulting in a less severe phenotype than non-mosaic DS sufferers (Devlin & Morrison, 2004). Using the transchromosomic approach more closely mirrors a full human trisomy, and does not interrupt endogenous mouse sequences. Tc1 mice have been shown to successfully age beyond two years with no signs of debilitation. Tc1 mice show reduced long-term potentiation (LTP) in the hippocampal dentate gyrus region (O'Doherty et al., 2005) and impaired performance on tasks such as object recognition memory. However, unlike individuals with DS, Tc1 mice are not functionally trisomic for APP (Gribble et al., 2013) and thus they provide an opportunity to evaluate the contribution of chromosome 21 genes to cognition in the absence of APP-related brain changes. Tc1 mice possess many of the phenotypic characteristics of human Down syndrome, including alterations in heart development, mandible size and neuronal number, as well as exhibiting some impaired memory performances (O'Doherty et al., 2005).

1.2.6. Tc1 phenotype

Because a primary aspect of DS is learning difficulties, Tc1 mice were initially assessed for potential cognitive deficits using behavioural tests of learning and memory, and by measuring hippocampal LTP. Field recordings of evoked potentials in the dentate gyrus of the hippocampus suggested normal basal synaptic transmission, however, Tc1 mice exhibited significantly reduced LTP in the dentate gyrus, when compared with wild-type littermates (O'Doherty et al., 2005). This result was furthered by Morice et al. (2008) who examined hippocampal LTP in the dentate gyrus of freely-moving mice, and followed both early and late LTP over several days. One hour after tetanic stimulation of the medial perforant pathway, Tc1 mice exhibited significantly reduced LTP compared with that of WT littermate controls. However, following both 24 and 48 h, LTP was comparable in both Tc1's and WT's. Morice et al., (2008) also provided evidence for a reduction in the expression of the GluR1 subunit of the AMPA receptor, which may be linked to a reduction in early LTP in Tc1 mice.

When tested on the novel object recognition task, wild-type mice spent significantly more time exploring a novel object than familiar objects. Tc1 mice, however, failed to distinguish between novel and familiar objects (O'Doherty et al., 2005). Morice et al., (2008) furthered this finding by demonstrating that Tc1 mice displayed a deficit in object recognition memory following a delay of 10-min, but not following a 24-h delay; which supported the conclusion that Hsa21 expression impaired short- but not long-term memory. This is in support of the electrophysiological data, with LTP deficits being mirrored by behavioural deficits in terms of delay periods. However, it remains unclear whether the deficit in short-term memory in Tc1 mice extends to a different sensory modality and whether memory for the visuo-spatial attributes of objects is relatively proficient. The latter issue is relevant given evidence that Tc1 mice display aberrant hippocampal short-term, but not long-term, synaptic plasticity, abnormal hippocampal spine morphology, and sub-region changes in the connectivity of the DG-CA3 network that contributes to disruption of place-cell activity (O'Doherty et al., 2005; Witton et al., 2015). The presence of hippocampal synaptic and place cell deficits suggests that the contribution of this structure to place recognition memory in Tc1 mice should be impaired. Hippocampal abnormalities in Tc1 mice appear to transiently impair spatial working memory as tested by their performance on the radial arm maze (Witton et al., 2015).

When tested on the cued version of the MWM, no difference was observed between Tc1 and WT litter mate controls. The distance travelled to escape decreased significantly with time for all the mice, demonstrating that Tc1 mice have no impairment in forming associations. Tc1 mice were then tested for spatial learning and memory performance in

the Morris watermaze using the hidden platform version of the task. Acquisition was similar for Tc1 mice as it was for WT mice, with Tc1 mice and WT littermates swimming a similar distance to find the hidden escape platform. Equally, on the probe trial, Tc1 mice showed a strong preference for the target quadrant, where the platform had been located, demonstrating an intact ability of Tc1 mice to adopt a spatial navigation tactic to locate the platform. In contrast, when spatial WM was then evaluated in a series of learning-reversal tests in which the location of the hidden platform was changed each day, Tc1 mice showed a delay in the acquisition of the task, and Tc1 mice showed significantly impaired spatial WM and when monitoring the swim trajectories of the mice on the probe trial, Tc1 mice demonstrated clear deficiencies in the use of spatial strategies to solve the task.

1.3. Aims and Hypotheses

This chapter has provided an overview of the history, prevalence and genetic basis of DS, as well as providing a review of the cognitive symptoms which individuals with DS present with. The various mouse models of DS have been described, and it is clear that the available mouse models, although useful, are not true recapitulations of the genetics of the human condition. For this reason, efforts to create new models of trisomy-21 are still ongoing. The development of the Tc1 mouse (O'Doherty et al, 2005) has the potential to overcome some of the difficulties caused by other partial trisomy models; the mouse contains a freely segregating copy of the human chromosome 21, an approach which more closely mirrors a full human trisomy. To date, little work has been carried out regarding the cognitive phenotype of the Tc1 mouse. The major aim of this thesis was to investigate recognition memory, and processing in the MTL, of Tc1 mice as compared to WT mice.

Tc1 mice have been shown to display a deficit in object recognition memory following a delay of 10-min, but not following a 24-h delay (Morice et al., 2008; O'Doherty et al., 2005). The initial aim of this thesis therefore was to attempt to replicate this pattern of results, before going on to further probe other properties of recognition memory in the Tc1 mice. Given evidence for aberrant hippocampal morphology, plasticity and coding of place information (Witton et al., 2015) it followed that there would be some disruption of spatial memory processes. Chapter 3 investigated this hypothesis using tasks that revealed memory for the visuo-spatial attributes of objects in Tc1 and control mice. Chapter 4 further interrogated whether the problems with short-term recognition memory were apparent when information was retrieved from long term memory. Chapter 4 also examined potential changes in some brain regions which make up part of the recognition memory system, by measuring expression of the immediate early gene *c-fos* in the hippocampus and perirhinal cortex. Based on evidence for reduced expression of the GluR1 subunit of the AMPA receptor (Morice et al., 2008), chapter 5 tested the hypothesis that the short term object recognition memory deficit would be improved by administration of a novel, AMPA receptor positive allosteric modulator, drug 9A.

2. Colony Preparation and Biochemical Analyses Materials and Methods

Introduction

This chapter describes the generation and maintenance of the mouse colonies used in this thesis; both the Tc1 and Tg2576 lines. Following the generation of the mouse colonies, genotypic analysis was performed in order to identify the genotype of the mice as either wildtype, transchromosomic or transgenic (those harbouring the genetic mutation). General behavioural methods are described, however, any protocols that are specific to individual experiments are described in the relevant chapter. Cohort information and statistical methods are also described. Tc1 mice were bred, and genotyped by collaborators at The Francis Crick Institute, London. The methods for breeding maintenance and genotyping of the Tc1 line is described, but this work was not carried out by the author.

2.1 Colony Preparation

2.1.1 Generation and maintenance of the mouse lines

2.1.1.1 Generation of the Tc1 mouse line

The Tc1 mouse line was initially formed by generating transchromosomic chimeras. It was necessary to first generate a panel of transchromosomic male mouse embryonic stem (ES) cell lines, each carrying a freely segregating copy of human chromosome 21 (Hsa21). The transchromosomic ES cells were then injected into host blastocysts and the resulting chimeras were mated to mice from the C57BL/6J strain (O'Doherty et al., 2005). Germline transmission was achieved from one female chimera, carrying the transchromosomic ES cells. The first female chimera gave birth to two offspring, which both retained the freely segregating copy of Hsa21 (O'Doherty et al., 2005). Various different background strains were assessed, but the transmission rate of Hsa21 diminished and only the colony on the C57BL/6Jx129S8 background was retained.

2.1.1.2 Maintenance of the Tc1 mouse line

Tc1 mice (B6; 129S-Tc (HSA21)1TybEmcf) were then maintained by mating Tc1 females to F1 (129S8xC57BL/6) males, with an approximate 40% transmission rate of the progeny inheriting Hsa21. At approximately 6 weeks of age, litters were weaned and placed in same-sex cages consisting of 2-4 mixed-genotype littermates. Male Tc1 mice and their age-matched wild type (WT) male litter mates were bred, ear-marked and subsequently genotyped at the Francis Crick Institute, London, and transferred to Cardiff University, with appropriate legal/ Home Office documentation, at ~2 months of age.

2.1.1.3 Generation of the Tg2576 mouse line

Tg2576 mice over-express the human APP 695 gene containing the 'Swedish' mutation (Lys670 -Asn and Met 671 -Leu) driven by a hamster prion protein gene promoter on a hybrid C57B6/SJL background (Hsiao et al., 1996). The Tg2576 line was originally founded by a C57B1/6J x SJL F3, which was then twice crossed back into C57B1/6J (Hsiao et al., 1996). The colony has been maintained at Cardiff University since 1998.

2.1.1.4 Maintenance of the Tg2576 mouse line

In order to maintain the genetic contributions of each background and ensure overexpression of the hAPP transgene, subsequent breeding of the colony has involved crossing Tg2576 into a C57B1/6J x SJL FI line. In practice, the breeding of further generations has involved pairing a male Tg2576 mouse with a female C57B1/6J x SJL. At approximately 6 weeks of age, litters were weaned and placed in same-sex cages consisting of 2-4 mixed-genotype littermates. Mice were ear marked post-weaning for identification, and the resulting ear biopsy was placed immediately on dry ice before being used for genotyping, as described in section 3 of this chapter. The resulting offspring were either heterozygous Tg2576 transgenic (TG) mice, possessing the mutation, or homozygous Tg2576 wildtype (WT) mice, at a ratio of approximately 50:50.

2.1.1.5 Housing Conditions

Animals were housed in standard conditions in cages measuring L 48cm x W 15 cm x H 13cm with an opaque plastic base and a wire top. Each cage was lined with sawdust, and mice were provided with environmental enrichment in the form of cardboard nesting

tubes and wooden chew sticks. Mice were kept on a 12-h light/dark cycle (lights on at 07.00 to 19.00), and all behavioural testing was conducted during the light phase of the cycle. Animals were kept in a temperature and humidity controlled environment (temperature 21 ± 2°C and a humidity of 55% ± 10%) and were maintained on *ad libitum* access to food and water. Tc1 and WT litter mates were housed together in groups of 2–4 per cage. Although Tg2576 mice were weaned into groups, all Tg2576 and their WT littermates ended up being individually housed because of male aggression and the need to maximise survival rates. We acknowledge that individual housing, albeit through necessity, may have an impact on the behavioural phenotype of Tg2576 and WT mice. Nevertheless, the cognitive phenotypes reported previously and in the present study are similar to other published reports with this mouse line.

All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act (1986) and associated guidelines, as well as European Union directive 2010/63/EU. The programme of work was also approved by the local ethical review committee at Cardiff University, UK.

2.2 Genotyping

2.2.1 Genotyping of the Tc1 colonies

Genotyping of the Tc1 mice was carried out at the Francis Crick institute, London, prior to arrival at Cardiff University.

2.2.1.1 DNA Extraction in Tc1 tissue

DNA extraction was carried out using the HotSHOT method (Truett et al., 2000).

2.2.1.2 Polymerase Chain Reaction for Tc1 genotyping

Polymerase chain reaction (PCR) was performed to determine the presence of the Hsa21 chromosome in Tc1 mice. A primer mix was made (see table 2.1). A PCR master mix was made up consisting of 1µl of the Tc1 primer mix and 8µl of Megamix blue (Microzone), which contains recombinant Taq polymerase, MgCl2 buffer and dNTPs required for PCR. 9µl of PCR master mix was then added to 1µl of each DNA sample. PCR was performed with cycling steps as listed in table 2.2.

	Target	Primer Name	Primer Sequence	PCR Product Size (bp)
Tc1	Hsa21	D21S55F (20µl)	GGT TTG AGG GAA CAC AAA GCT TAA CTC CCA	208
		D21S55R (20µl)	ACA GAG CTA CAG CCT CTG ACA CTA TGA ACT	208
	Mouse Genome	MyoF (10µl)	TTA CGT CCA TCG TGG ACA GCA T	200
		MyoR (10µl)	TGG GCT GGG TGT TAG TCT TAT	200
Tg2576	APP*swe* transgene	1502	GTG GAT AAC CCC TCC CCC AGC CTA GAC CA	300
		1503b	CTG ACC ACT CGA CCA GGT TCT GGG T	300
	Endogenous prion protein	1501	AAG CGG CCA AAG CCT GGA GGG TGG AAC A	600

Table 2.1 Primer information for Tc1 and Tg2576 PCR. *All primers, their target, name, sequence from 5'→3', and their product size (base pairs).*

Tc1 PCR			Tg2576 PCR		
Step	Temperature	Time	Step	Temperature	Time
1	95˚C	3min	1	94˚C	45s
2	95˚C	30s	2	60˚C	30s
3	62˚C	60s	3	72˚C	1min 30s
4	72˚C	45s			
Repeat steps 1-4 x35			Repeat steps 1-3 x35 (each cycle +2seconds)		
5	74˚C	10min	5	72˚C	5min
Terminated and maintained at 4˚C					

Table 2.2 PCR cycling conditions for Tc1 and Tg2576 genotyping.

2.2.1.3 Gel electrophoresis for Tc1 genotyping

Following PCR, 2% agarose gels were made up by dissolving agarose (Invitrogen) in 1X TBE buffer (National Diagnostics) in a microwave at high power for 2 mins. The solution was allowed to cool before adding 0.05µg/ml of ethidium bromide (Sigma-Aldrich). The gel solution was then poured into a gel cast and allowed to set. Samples were loaded alongside molecular weight marker HyperLadderTM 100bp (Bioline). Gel electrophoresis was performed in 1X TBE (National Diagnostics) at 120V for 1hr 30 mins. Products of the PCR reaction were then visualised in an illuminator under UV light, and images of the results were acquired.

2.2.2 Genotyping of the Tg2576 colony

Ear clips were taken from mice post-weaning in order to aid with identification; the resulting tissue biopsies were then used to extract DNA, and subsequent PCR analysis was carried out. PCR uses specific oligonucleotide primers to selectively amplify specific deoxyribonucleic acid (DNA) regions of interest, in order for them to be measured using gel electrophoresis.

2.2.2.1 DNA extraction in Tg2576 tissue

In preparation for PCR, ear tissue from the mice was digested, and DNA was extracted using the DNeasy blood and tissue kit (Qiagen, UK). Firstly, the incubator was preheated to 56^{o}C. Next, 20 µl proteinase K stock solution and 180 µl Buffer ATL was added to each sample, and mixed by vortexing. After ensuring that the tissue samples were fully submerged in the proteinase K – Buffer ATL mix, the samples were incubated at 56^{o}C until fully lysed, vortexing occasionally during the incubation period. Next, 200µl of Buffer AL, and 200µl of ethanol were added to each sample, before pipetting the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. The samples were then centrifuged at ≥ 6000 x *g* (8000 rpm) for 1min, and the flow-through and collection tubes were discarded, before placing the spin columns in new 2 ml collection tubes. Next, 500 µl of Buffer AW1 was added to the spin column of each sample, and centrifuged for 1 min at 8000rpm. Again, the flow-through and collection tubes were discarded, and the spin columns were placed in new collection tubes. Next, 500 µl of Buffer AW2 was added to the spin column of each sample, and centrifuged for 3 min at 20,000 x *g* (14,000 rpm). The flow-through and collection tubes were discarded for a final time, and the spin columns were now transferred to new 1.5 ml microcentrifuge tubes. The DNA was eluted by adding 200 µl Buffer AE to the centre of the spin column membrane, which was incubated at room temperature (~25^{o}C) for 1 min before being centrifuged for 1 min at

8000rpm. The spin columns were then discarded, and the pure DNA samples were contained in the 1.5 ml microcentrifuge tubes. PCR was then either immediately carried out on the samples, or the samples were stored at -20^{o}C until required for PCR.

2.2.2.2 Polymerase Chain Reaction for Tg2576 genotyping

To carry out the PCR, 2.5µL of DNA was added to 22.5µL of Master Mix on ice in DNase, RNase-free aliquots. A 22.5µL measure of Master Mix contained 16.625µL nuclease free water (Fisher), 2.5µL 10x PCR Buffer (InVitrogen, Paisley, UK), 1.25µL 50mM $MgCl_2$ (InVitrogen, UK), 1.25µL deoxynucleotide tri-phosphates (dNTPs, GE Healthcare, Little Chalfont, UK), 0.025µl of primer 1501 at a concentration of 50pmol, 0.025µl of primer 1502 at a concentration of 10pmol, 0.025µl of primer 1503b at a concentration of 10pmol and 0.125µL of Taq polymerase (1µg/µl, InVitrogen, UK). Oligomer primers used for amplifying and detecting the APPswe transgene were 1503b, and 1502, while a probe for the endogenous murine prion protein required Primers 1502 and 1501 (see table 2.1).

Samples were immediately transferred from ice to a thermocycler (MJ Research, Massacheusettes, USA) and PCR was performed with cycling steps as listed in table 2.2.

2.2.2.3 Gel electrophoresis for Tg2576 genotyping

DNA products were separated by gel electrophoresis on a 1% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0, Pierce) in an ethidium bromide-free docking system (Bio-Rad, Hertfordshire, UK). Initially, 15µL of DNA product was mixed with 3µl of “Novel Juice” loading dye (GeneDirex, Newmarket, UK). Samples were run alongside a 100bp DNA ladder (GeneDirex), a positive control TG control and a negative WT control from mice of confirmed genotypes, and one water blank control, whereby DNA was replaced with nuclease free water. The water blank control was used as a means of detecting potential contamination (see Figure 2.1). Samples were run at 100V for 1 hour, and products were visualised using a canon digital camera, and Alpha DigiDoc software, and sized against the DNA ladder. A single band at ~600 bp corresponds to amplification of the endogenous murine prion protein gene, which should be present in all tissue samples from Tg2576 transgenic and WT mice. The presence of the second band at ~300-bp fragment represents the HuAPP695 region with the K670N, M671L APP transgene, which confirms the tissue sample was collected from a TG mouse.

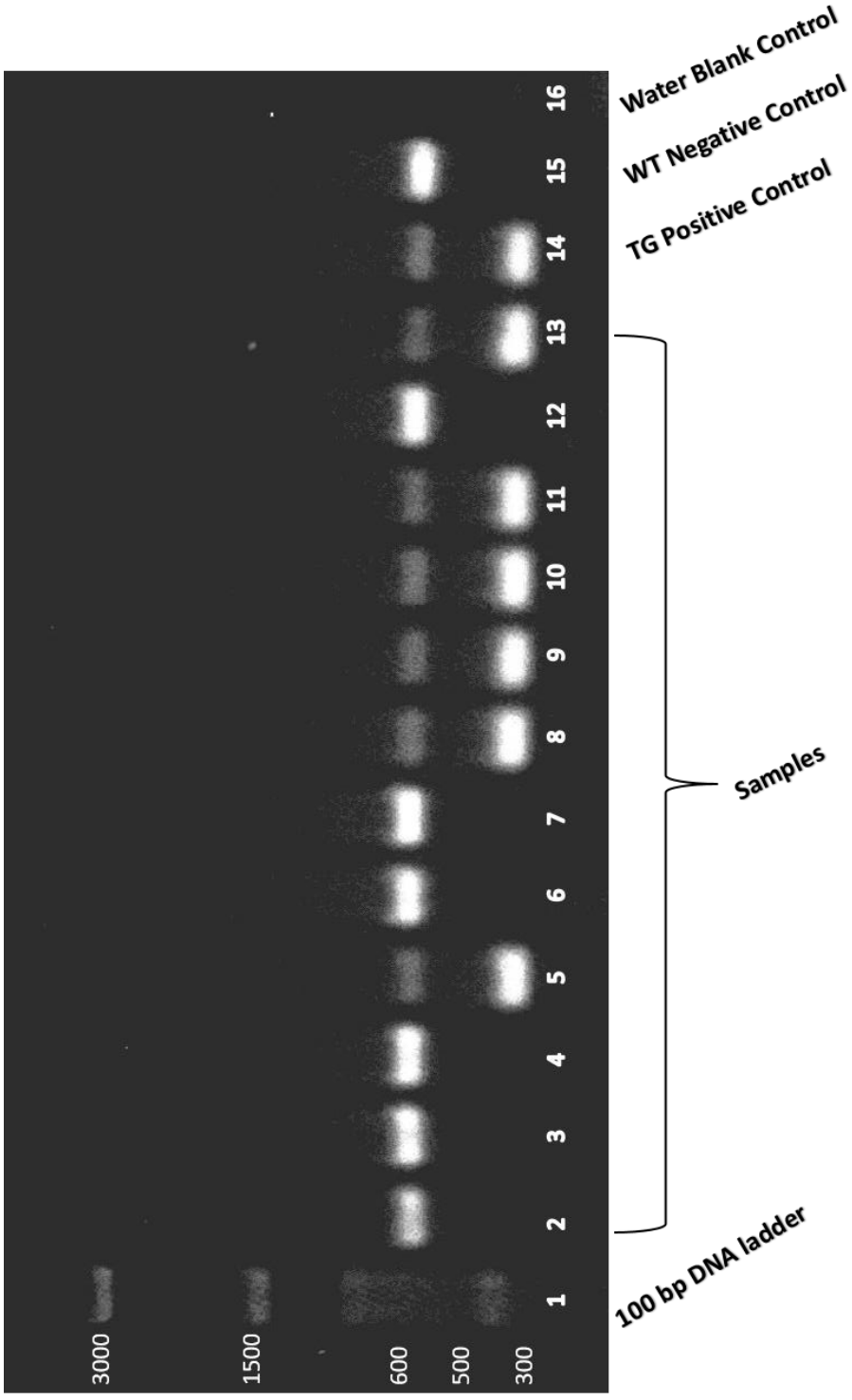


Figure 2.1 Gel electrophoresis from Tg2576 PCR. *Representative photograph of gel electrophoresis showing PCR amplification of DNA from Tg2576 and WT mice. Lane 1 = 100bp DNA ladder; Lanes 2, 3, 4, 6, 7 and 12 show a single band corresponding to amplification of endogenous murine prion protein, thus representing WT mice; Lanes 5, 8, 9, 10, 11 and 13 show a double band corresponding to amplification of endogenous murine prion protein and APPswe transgene, therefore representing TG mice; Lane 14 shows the positive control (known TG sample), Lane 15 shows the negative control (known WT sample), and lane 16 shows the water blank control, containing no DNA.*

2.3 Characterising the behavioural phenotype of Tc1 mice

A full scientific justification for the use of these behavioural tasks, and a more thorough description of each behavioural protocol will be reserved for the appropriate empirical chapters, here, only cohort information and the basic set-up and procedures are described.

2.3.1 Experimental cohorts

Four cohorts of male Tc1 (Transchromic Tc1 and WT littermates) mice were bred at the Francis Crick Institute, London, transferred to Cardiff University, with appropriate legal documentation, at ~2 months of age and used in the experiments described in this thesis. Only male mice were used throughout this thesis. Gender differences in mice have been regularly noted, and this can ultimately have an effect on the resulting behaviours of the two genders. Male mice produce significantly higher amounts of testosterone from their testes, than female mice produce from their ovaries, and this difference in testosterone level can result in sexual dimorphisms in the nervous system (Crusio, Sluyter, Gerlai and Pietropaolo, 2013). The resulting structural differences in the nervous systems of the different genders of mice is thought to account for some differences in behaviour (Morris, Jordan and Breedlove, 2004). In addition, adult hormone effects are differentially present in male and female mice; after puberty, gonadal hormone production becomes more regular, and rhythms in hormone production are observed, particularly in females (Crusio, Sluyter, Gerlai and Pietropaolo, 2013). Adult females have a surge in oestradiol and progesterone production following ovulation, which occurs every four to five days; these surges in hormone production can also induce changes in behaviour (Crusio, Sluyter, Gerlai and Pietropaolo, 2013). Thus, overall, the inclusion of both male and female mice could increase variability due gender related differences in behaviour, so much so that a result is undetectable. The effect of trisomy-21 on females is a question which cannot be ignored, but which would be better addressed when group size is large enough that gender can be considered as a between subjects factor. Further details of the number, age and order of experiments which each group participated in can be found in Table 2.3. The exact number of mice used in each task can be found in the methods section of experimental chapters, as due to attrition rates these varied between experiments.

Cohort	Total Animals	WT	Tc1	Experiment	Age at Testing	Thesis Chapter
Cohort 1	26	13	13	NOR 10 min v 24 hr	4-7months	3
	24	12	12	Context v Objects	5-8months	4
				Context 10 min v 24 hr	5-8months	4
	20	10	10	*c-fos*	6-9months	4
Cohort 2	16	8	8	NOR 10 min v Immediate	4-7months	3
				OinP	5-8months	3
				OL	5-8months	3
				GluR1, GRIK1, GRIK5 Immuno	12months	5
Cohort 3	24	12	12	Novel Odour	4-7months	3
Cohort 4	24	12	12	NOR 10min v 40min	5months	5
				AMPAkine	10months	5
				Modafanil	10months	5
				Temporal Order	15months	3
Cohort	Total Animals	WT	Tg2576	Experiment	Age at Testing	Thesis Chapter
Cohort 1	21	10	11	NOR 10 min v 24 hr	10-11months	3

Table 2.3 Information regarding experimental cohorts. *Number, genotype and age of animals in each of the four experimental Tc1 cohorts, and one experimental Tg2576 cohort used for experiments described throughout this thesis. Details of each of the tasks each cohort participated in are listed, along with the chapter of the thesis in which the full description and results of the tasks can be found.*

2.3.2 Recognition memory tasks

All behavioural tasks used exploited natural rodent behaviours. All recognition memory tasks (novel object recognition, object in place, object location and olfactory recognition) require the same apparatus and general set-up which will be described here.

2.3.2.1 Apparatus

The apparatus used for all object exploration experiments was a large Perspex arena, 60 x60 x40 cm, with a white floor and clear walls. The box was placed on a square table at waist height. The apparatus was set up in a quiet and brightly lit (38 cd/m^2 at the arena surface) behavioural testing room as shown in Figure 2.2. Exploration was recorded with an overhead camera. The camera input was used to monitor activity in the arena on a television monitor and each session was recorded using a Philips DVDR recorder. The duration of object exploration throughout the trials was recorded manually with a stopwatch. All objects used were everyday objects made of non-porous materials. All objects were at least 10 cm high to avoid the mice climbing and sitting on the objects, and were all weighted so that they could not be displaced by the animals. A selection of objects used can be seen in Figure 2.3. Both the arena and the objects (including novel objects) were cleaned thoroughly with water and ethanol wipes in between each trial in order to prevent the use of odour cues, urine and excrement were also removed from the arena after each trial.

For the olfactory recognition experiment, odour cubes were purchased from Dale Air Ltd (UK) (see Figure 2.4). Odour cubes were 5 x5 x5cm and red in colour with holes placed in one surface. The scents used were strawberry, coconut, banana, lime, mint, ginger, cinnamon and coriander.

2.3.2.2 Experimental Design

The week prior to testing, mice were handled for 5 min a day. For three days prior to testing, mice were placed in the behavioural test room in their home cages, for 30 min a day. On the fourth day, mice were also given one habituation session in which to freely explore the arena with no objects present for 10 min. Training commenced the following day. For olfactory recognition, mice were habituated to the odour cubes by placing unscented versions of the cubes in their home cages for three days prior to testing. The order of presentation of experimental conditions, and the spatial location of objects was counterbalanced amongst mice in order to avoid order effects or spatial biases; this will be described in more detail in the relevant chapters. For each experiment, the dependent

variable was the amount of time spent by the animals exploring objects. Object exploration was defined as the time spent attending to (actively sniffing or interacting with) the object at a distance no greater than 1 cm. Object exploration was not scored if the animal was in contact with but not facing the object, or if it attempted to climb on the objects to look around the rest of the arena. In order to ensure that procedures were sensitive to differences between the groups independent of variation in individual contact times, a discrimination ratio was calculated for each experimental test phase and these are described in the appropriate methods section. A value close to 1 indicated a strong preference for the target object, whereas a value of 0.5 indicated no systematic bias for the target object.

Figure 2.2 - behavioural testing room set up. *The apparatus used for all object exploration experiments was a large Perspex arena, 60 x60 x40 cm, with a white floor and clear walls. The box was placed on a square table at waist height. The apparatus was set up in a quiet and brightly lit (38 cd/m² at the arena surface) behavioural testing room.*

***Figure 2.3 – objects**. A selection of* objects used in the novel object recognition and spatial recognition tasks.

***Figure 2.4 - odour cubes.** Odour cubes from Dale Air Ltd, UK. All cubes were 5 x5 x5cm and red in colour with holes placed in one surface. The scents used were strawberry, coconut, banana, lime, mint, ginger, cinnamon and coriander.*

2.4 Data Analysis

All data was recorded in spreadsheets using Microsoft Excel, which was then used to calculate discrimination ratios, means, standard deviations and standard error of the mean. All subsequent statistical analysis was carried out using IBM SPSS (version 20.0).

2.4.1 Statistical Analysis

Parametric statistical analyses were carried out primarily using an analysis of variance (ANOVA) or t-tests. The a-level was set at $p < 0.05$ for all comparisons. In order to use t-test and ANOVA, it is essential that the dependent variable is measured on a continuous level (they are interval or ratio variables), and that variables are independent of each other; these things were ensured when designing the experiments. It is also essential that there are no significant outliers; this was checked using SPSS prior to carrying out subsequent analysis. The remaining assumptions of t-test and ANOVA were tested in SPSS, including normal distribution, homogeneity of variance and sphericity.

Normality was tested in SPSS using the Shapiro-Wilk test; if this assumption was violated ($p<0.05$) then the kurtosis value was checked. ANOVA is fairly robust against violations of normality, except when platykurtosis is present (Kinnear and Gray, 2009). However, in scenarios where the assumption of normality was violated then non-parametric tests were used (Kruskal Wallis test used for within-subjects designs).

The assumption of homogeneity of variance was tested in SPSS using the Levene's test of equality of error variances. In scenarios where the Levene's test was significant ($p<0.05$) then the assumption of homogeneity of variances was violated, and non-parametric tests were used (Kruskal Wallis test used for within-subjects designs).

Finally, the assumption of sphericity was tested in SPSS by using the Mauchly's test of sphericity, and if this assumption was violated ($p<0.05$) then an epsilon correction was made by reporting the Greenhouse-geisser result in the SPSS output table.

2.4.2 Estimating adequate sample size: the “resource equation”

The use of too many animals is unethical. However, if too few animals are used the experiment may lack power and miss a scientifically important response, which is equally unethical. As the Tc1 mice were new to our lab, it was not possible to make assumptions about effect size, or have an accurate idea about standard deviations within groups. Thus, it was difficult to use power calculations to determine an appropriate sample size. In addition, due to the fact that breeding of animal cohorts was not under our own control, we often had little influence over the size of cohorts. However, using the “resource equation” method (Charan & Kantharia, 2013), we were able to crudely estimate whether the number of animals within a cohort was likely to be sufficient, so that we could request more animals at accurate time points, and only when needed, so as to fall in line with the principles of the 3R’s (replacement, reduction and refinement).

According to this method, which is based on ANOVA, a value “E” is measured. If E is less than 10 then adding more animals will increase the probability of getting significant results, but if it is more than 20 then adding more animals will not increase the probability of getting significant results. Thus, any sample size, which keeps E between 10 and 20 should be considered as an adequate number of animals to be using. E is calculated by taking the total number of animals, and subtracting the total number of experimental groups.

So for example, cohort 1 consists of 26 animals in total (see table 2.3), and in all experiments cohort 1 participated in, there are two groups (WT and Tc1). Thus E = 26 – 2 = 24. Therefore adding more animals to this cohort will not increase the chance of statistically significant results. The number of animals is slightly higher than necessary, but allows for attrition.

This method cannot be considered as robust as power analysis, but provided a loose method of estimating adequate sample sizes in the given scenario.

3. Tc1 mouse model of trisomy-21 dissociates properties of short- and long-term recognition memory

Chapter Overview

This chapter examines memory function in Tc1 mice, a transchromosomic model of Down syndrome (DS). Tc1 mice demonstrated an interesting delay-dependent deficit in recognition memory. More specifically, Tc1 mice showed intact immediate (30 sec), impaired short-term (10-min), and intact long-term (24-h) memory for objects. A similar pattern was observed for olfactory stimuli, confirming the generality of the pattern across sensory modalities. The specificity of the behavioural deficits in Tc1 mice was confirmed using APP overexpressing mice that showed the opposite pattern of object memory deficits. Tg2576 mice express a human Swedish APP mutation linked to early onset Alzheimer's disease. A comparison between the Tc1 and Tg2576 mice was also of theoretical interest because the overexpression of APP is absent in Tc1 mice, unlike Down syndrome individuals (and other DS mouse models). In contrast to object memory, Tc1 mice showed no deficit in either immediate or long-term memory for object-in-place information. Similarly, Tc1 mice showed no deficit in short-term (10 min delay) memory for object-location information. The latter result indicates that Tc1 mice were able to detect and react to spatial novelty at the same delay interval that was sensitive to object novelty recognition. These results demonstrate (1) that novelty detection *per se* and (2) the encoding of visuo-spatial information was not disrupted in adult Tc1 mice. This chapter concludes that the task specific nature of the short-term recognition memory deficit suggests that the trisomy of genes on human chromosome 21 in Tc1 mice influenced perirhinal cortical systems supporting short-term object and olfactory recognition memory.

3.1 Introduction

The Tc1 mouse model of trisomy-21 has previously been shown to display a deficit in object recognition memory following a delay of 10-min, but not following a 24-h delay (Morice et al., 2008; O'Doherty et al., 2005). This observation supported the idea that Hsa21 expression impaired short- but not long-term memory. However, it remains unclear whether the deficit in short- term memory in Tc1 mice extends to a different sensory modality and whether memory for other aspects of the properties of objects, for example, their visuo-spatial attributes, is influenced in a similar fashion.

The medial temporal lobe (MTL, which consists of the hippocampus, which can be divided into the CA1, CA2, CA3 and DG subfields, along with the perirhinal cortex, the postrhinal cortex and the lateral entorhinal cortex and the medial entorhinal cortex) plays a major role in recognition memory (Brown & Aggleton, 2001; Squire et al., 2007). It has been demonstrated that individuals with DS have difficulty in performing cognitive tasks that map onto the putative functions of MTL structures. This chapter will first consider the anatomical substrates of recognition memory in normal rodents and its implications for our understanding of memory systems in Tc1 mice.

Recognition memory is a subcategory of declarative memory, and is ultimately the ability to recognise previously encountered events or objects (Brown & Aggleton, 2001). Recognition memory can be subdivided into two component processes: recollection and familiarity, sometimes referred to as "remembering" and "knowing", respectively (Mandler, 2008). When an item or object is encountered following an initial period of exposure, it is recognised as familiar, i.e., no longer novel. Familiarity involves knowing an item has been encountered before, whereas recollection involves remembering associated contextual information related to the familiar item (Brown & Aggleton, 2001). Whether recollection and familiarity are separate or unitary processes is a source of debate (Slotnick, 2013). This recollection-familiarity distinction was introduced by George Mandler (1980), and is more formally known as the dual process theory, which assumes that recognition is composed of two types of memory; namely, recollection and familiarity (Atkinson & Juola, 1974; Hintzman & Curran, 1994; Jacoby, 1991; Jacoby & Dallas, 1981). Recollection is a relatively slow process that consists of retrieving specific details associated with the prior presentation of an item, whereas familiarity is a relatively fast process that allows one to appreciate the fact that the item was previously encountered even though no contextual detail can be retrieved (Wixted, 2007). Full recognition involves knowing the answer to both familiarity and recollection based judgements; in the absence of familiarity information, recollection may provide some approximation of recognition (Mandler, 2008). A common criticism of dual process

models of recognition is the idea that recollection is simply a more vivid version of familiarity. A study by Rutishauser, Schuman & Mamelak (2007) reported that hippocampal neurons, regardless of successful recollection, responded to the familiarity of objects. However, the neuronal activity evoked by the presentation of a familiar stimulus differed between stimuli that were to be successfully recollected from stimuli that were not recollected. Thus, rather than consisting of two separate categories, single-process models regard recognition memory as a continuum ranging from weak memories to strong memories, i.e., the stronger the neuronal response, the better the memory (Rutishauser, Schuman & Mamelak, 2007).

The specific roles of the MTL in recognition memory remains a topic of controversy. Nevertheless, there is a general consensus that the MTL contributes to recognition memory (Brown & Aggleton, 2001; Squire et al., 2007). Advocates of the dual-process model of recognition memory argue that recollection and familiarity have distinct neural substrates, with different regions of the MTL making different contributions to different aspects of recognition memory (Eichenbaum, Yonelinas & Ranganath, 2007). It has been argued for example, that although the hippocampus is essential for episodic and recollective memory, it is not necessary for familiarity-based recognition (Eichenbaum, 2004); this view predicts that patients with selective hippocampal pathology can still use their intact familiarity system to guide recognition and thus show sparing on some tests of recognition (Aggleton & Brown, 1999). These multi-functional models usually then assume that the perirhinal cortex for example, independently of the hippocampus, is the structure which is vital for familiarity based recognition (Brown & Aggleton, 2001). The single process model of recognition memory suggests that the hippocampus is just as essential for recognition as it is for recall; that both aspects of memory are intrinsic components of the same class of memory (Knowlton & Squire, 1995). This model suggests that the contributions of different regions of the MTL cannot be separated, and whereby the hippocampus receives convergent information from all of the medial temporal lobe and ultimately heads up a 'medial temporal lobe memory system' (Squire & Zola-Morgan, 1991). According to the single process model, the hippocampus is vital for all forms of declarative memory (Squire, 1992).

The assessment of recognition memory in rodents is often carried out using spontaneous preference tests. Such tests exploit rodent's innate preference for exploring novel objects or arrangements of objects. For example, following exposure to two copies of object "A" in an open arena, rodents will show a preference for exploring a novel object "B" during a test phase when presented simultaneously with the familiar object (Ennaceur & Delacour, 1988). This simple protocol has been used extensively, is robust, and well replicated across a range of conditions, parameters and rodent species (Ennaceur,

Neave & Aggleton, 1997; Dix & Aggleton, 1999; Norman & Eacott, 2005; Good and Hale, 2007; Langston & Wood, 2010).

It is generally acknowledged that a major contribution of the hippocampus to recognition memory is processing object location and context information (Barker & Warburton, 2011). In contrast, there is relatively little evidence that the hippocampus contributes to short-term object memory (Hammond, Tull, & Stackman, 2004). Many studies have reported no effect of hippocampal lesions on tests of novel object recognition (Aggleton, Hunt, & Rawlins, 1986; Barker & Warburton, 2011; Forwood, Winters, & Bussey, 2005; Kesner, Bolland, & Dakis, 1993; Mumby, Pinel, Kornecook, Shen, & Redila, 1995; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). However, some studies of hippocampal lesions demonstrate a deficit on object recognition tasks that are thought to be as a result of aberrant hippocampal function (Broadbent, Squire, & Clark, 2004; Cohen et al., 2013; Gaskin, Tremblay, & Mumby, 2003). Despite some controversy, one study has demonstrated that the recognition memory deficit following hippocampal lesions is much less severe than the deficit caused by perirhinal lesions (Prusky, Douglas, Nelson, Shabanpoor, & Sutherland, 2004). In addition, temporary inactivation of the hippocampus in both mice and rats reduced performance on the novel object recognition task, although the discrimination still remained above chance levels (Hammond, Tull and Stackman, 2004). Evidence from Clarke, Zola and Squire (2000) also demonstrated a delay dependent deficit in recognition memory following hippocampal lesions in rats; memory was assessed at five different delay intervals ranging from 10 sec to 24-hrs, and all groups performed normally at the shorter delays (10 sec and 1 min). However, across longer delays, the groups with hippocampal damage were impaired, suggesting a role for the hippocampus in recognition memory under longer delay conditions.

Although some studies suggest a role for the hippocampus in recognition memory, many studies have suggested that this role may not be for the recognition of objects *per se*, but that the hippocampus plays a role in other aspects of recognition memory, such as the representation of the spatio-temporal context. Several studies have reported that hippocampal lesions cause deficits in spatial memory tasks while leaving object recognition memory unaffected (Aggleton, Hunt & Rawlins, 1986; Duva et al., 1997; Jackson-Smith, Kesner, & Chiba, 1993).

In contrast, lesions of the perirhinal cortex consistently impair object recognition memory in rats when tested on the spontaneous object recognition paradigm (Aggleton, Keen, Warburton, & Bussey, 1997; Ennaceur & Aggleton, 1997; Ennaceur, Neave, & Aggleton, 1996). Similar results have been reported for olfactory and tactile recognition memory,

implicating the perirhinal cortex in recognition memory for a number of sensory domains (Suzuki, Zola-Morgan, Squire and Amaral, 1993).

Winters et al. (2004) found a clear double dissociation between perirhinal and hippocampal lesion groups. Rats with hippocampal lesions were impaired on the radial arm maze, while rats with perirhinal lesions performed at a comparable level to control animals. The rats with perirhinal lesions however were impaired on spontaneous object recognition, whereas those with hippocampal lesions were not impaired (Winters et al., 2004). Evidence consistent with a functional double dissociation between the perirhinal cortex and the hippocampus has also been provided by imaging experiments using the products of the immediate early gene *c-fos* as a measurement of neuronal activation. Wan, Aggleton and Brown (1999) reported that the perirhinal cortex was activated significantly more by novel images than familiar ones. In contrast, the hippocampus was not differentially activated by these manipulations. On the other hand, pictures of novel spatial arrangements activated the CA1 region of the hippocampus significantly more than pictures of familiar spatial arrangements, and the perirhinal cortex was not sensitive to these changes. This is suggestive of an important role for the perirhinal cortex in the representation of object information, and a role for the hippocampus in the representation of spatial information.

Disconnection analyses also shed light on the functional interaction between the perirhinal cortex and the hippocampus. For example, if the perirhinal cortex is lesioned in one hemisphere, and the hippocampus is lesioned in the contralateral hemisphere (Barker & Warburton, 2011), a functional connection between the perirhinal cortex and the hippocampus is not required for object recognition memory *per se* but is necessary for successful object-in-place memory and object recency memory (Barker & Warburton, 2011; Jo & Lee, 2010). Although a consensus has not been reached, studies suggest that the perirhinal cortex and the hippocampus work in parallel to support object recognition memory. However, the perirhinal cortex is capable of supporting recognition memory alone if required, whereas the hippocampus is not capable of supporting object recognition memory in isolation from the perirhinal cortex.

Electrophysiological and behavioural evidence suggests that the medial prefrontal cortex (mPFC) also contributes to recognition memory. mPFC neurons have been shown via single unit recordings to carry information concerning the relative familiarity of stimuli, with many units being active and signalling stimulus familiarity up to 24-hrs after presentation of the stimulus (Xiang & Brown, 2004). Damage to the mPFC has also been shown to impair recognition memory tasks (Bachevalier & Mishkin, 1986; Kolb, Buhrmann, McDonald, & Sutherland, 1994; Meunier, Bachevalier, & Mishkin, 1997). The mPFC has also been shown to play an important role in recency discriminations for

objects or spatial locations (Hannesson, Howland, & Phillips, 2004; Mitchell & Laiacona, 1998).

The MTL in Down Syndrome

Nadel and Pennington (2003) actually proposed the hippocampal hypothesis of DS, suggesting that cognitive impairment in DS is disproportionately influenced by hippocampal dysfunction. DS individuals show poor performance on spatial navigation, spatial recognition and object recognition tasks, (Pennington, Moon, Edgin, Stedron, & Nadel, 2003), which suggests that MTL functions are compromised. DS individuals also show difficulty with tasks of executive function, which map to the PFC (Lanfranchi, Jerman, Dal Pont, Alberti, & Vianello, 2010), however, recency memory in DS is relatively under studied. For example, DS individuals are impaired on the CANTAB pattern recognition memory test. DS participants had greater difficulty in recognizing a previously presented pattern than their age matched controls (Pennington, Moon, Edgin, Stedron & Nadel, 2003). In addition, DS participants performed significantly worse than the age matched control group on the CANTAB paired associate learning task. However, not all studies have consistently found problem with visuospatial memory; Courbois et al. (2013) used a wayfinding behaviour task with DS adults in a virtual town containing three target buildings and a number of visual landmarks, and were asked to learn two different routes. All chronologically age matched controls, 9/10 mentally age matched controls, and 7/10 participants with DS were successful in learning the two routes.

In contrast to Courbois et al (2013), Lavenex et al. (2015) used a physical navigation task in which participants were required to find three rewards hidden among 12 potentially rewarded locations in a 4m x 4m arena. Rewards were either located next to local cues (marked by a red cup), or only on the basis of their relationship to distal environmental cues. All age-matched controls found the rewards at above chance level both in the presence and the absence of the local cues. In contrast, all but one of the participants with DS exhibited a preference for the rewarded locations when local cues were present, whereas only 50% of participants with DS chose the rewarded locations at above chance level in the absence of local cues. This demonstrates that individuals with DS are impaired at using distal allocentric cues to navigate accurately in a real-world environment. The latter finding is consistent with the view that hippocampal function is compromised in DS patients.

Mouse models of DS permit exploration of the effects of gene triplication on cognitive systems at the behavioural systems and biological levels. The Tc1 mouse model of trisomy-21 has been shown to display a deficit in object recognition memory following a delay of 10-min, but not following a 24-h delay; which supports the idea that Hsa21

expression impaired short- but not long-term memory (Morice et al., 2008; O'Doherty et al., 2005), and spatial working memory (Witton et al., 2015). There is also evidence that Tc1 mice show aberrant hippocampal short-term, but not long-term, synaptic plasticity (LTP), abnormal hippocampal spine morphology, and sub-region changes in the connectivity of the DG-CA3 network that contributes to disruption of place-cell activity (O'Doherty et al., 2005; Witton et al., 2015). These observations provide support for the view that cognitive functions supported by the hippocampus are impaired in DS models and further predict that memory for the spatial organisation of objects will be disrupted in Tc1 mice (Burke et al., 2011; Lenck-Santini, Rivard, Muller, & Poucet, 2005). An important distinction to make at this stage between the Tc1 mouse and DS individuals, is that unlike Down syndrome individuals, the overexpression of APP is absent in Tc1 mice. APP plays a major role in brain development and neurogenesis and APP trisomy may contribute to abnormal brain development and cognition (Cataldo et al., 2003, Giacomini et al., 2015 and Trazzi et al., 2013). In this context, it is interesting to note that Tg2576 mice show intact short term recognition memory, and a deficit in long term recognition memory, a pattern similar to that shown by Ts65Dn mice (Good & Hale, 2007). Tg2576 mice express a human Swedish APP mutation linked to early onset Alzheimer's disease. To determine whether the pattern of recognition memory changes in Tc1 mice was specific to expression of Hsa21 and not a non-specific consequence of human gene expression, and to examine further the impact of APP overexpression on recognition memory, the performance of aged Tg2576 mice on the same behavioural procedure was also examined.

Therefore, the aim of this first experimental chapter was to probe the integrity of the MTL system in the Tc1 mouse model of trisomy-21: due to the interactive nature of this system, it is important to tease apart the ability of the Tc1 mice to carry out tasks which have been shown to be dependent on particular aspects of this system. First, we examined immediate, short and long-term recognition memory in Tc1 mice for both visual and olfactory information to test the hypothesis that Tc1 mice will show impaired short term but intact long term object recognition memory. Second, we tested the hypothesis that memory for the spatial organisation of objects will be impaired inTc1 mice. Finally, memory for the temporal order of objects was examined in Tc1 mice to explore the role of the prefrontal cortex as part of the interactive MTL system in these animals, with the hypothesis that there would be a deficit on tests for temporal order memory in the Tc1 mice.

3.2 Materials and Methods

3.2.1 Subjects

Four separate cohorts of animals were used in the current study. Experiment 1a was conducted with a cohort of 26 animals (13 WT and 13 Tc1 mice), aged 4-7months. However attrition led to only 12 WT mice reaching the end of the experiment; results therefore are from 25 mice (12 WT and 13 Tc1). Experiments 1b, 2a and 2b were conducted on a new cohort of 16 animals (8 WT and 8 Tc1). Mice were approximately aged 4-7 months for experiment 1b, and aged 5-8 months during experiments 2a and 2b. The interval between experiments 2a and 2b was approximately one week. Experiment 3 was conducted on a new cohort of 24 animals (12 WT and 12 Tc1 mice) and was conducted when animals were 4-7 months old. Experiment 5 was conducted on an older cohort of 24 mice (12 WT and 12TG), who were tested at 15 months of age. Experiment 4, used 11 heterozygous male Tg2576 mice that expressed the ‘‘Swedish” amyloid precursor protein mutation (HuAPP695SWE; driven by a hamster prion protein promoter; (Hsiao et al., 1996) together with 10 WT male litter mate control mice; these mice were tested at 10-11 months of age.

3.2.2 Apparatus

As described in Chapter 2.

3.2.3 Experimental design

As described in Chapter 2.

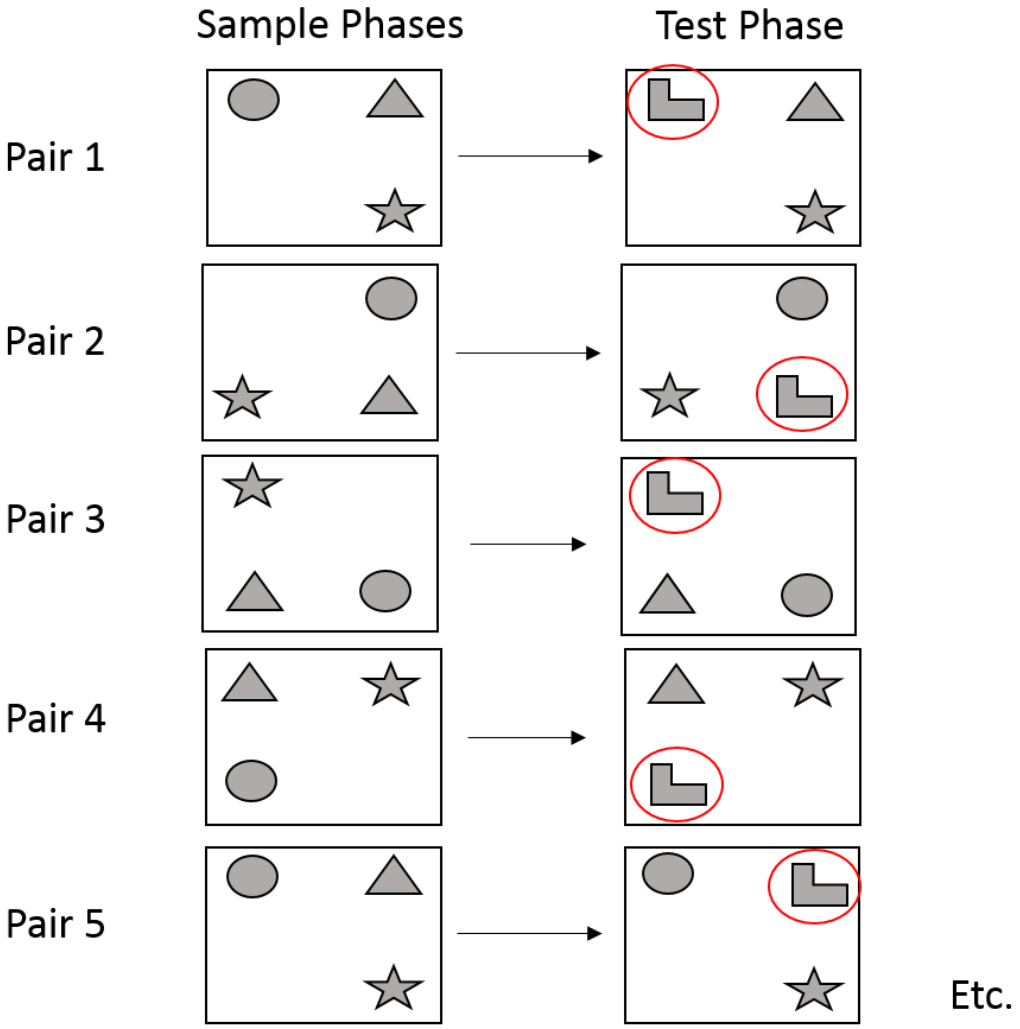


Figure 3.1 - Schematic representation of counterbalancing *of the spatial location of objects, and the item which was removed, for each pair of mice. The order in which experimental delay conditions were presented was also counterbalanced.*

3.2.4 Behavioural Methods

3.2.4.1 Experiment 1a: Tc1 novel object recognition following a 10-min or 24-h delay

Mice were placed in the centre of the arena and presented with three different objects, each in a different corner of the arena. Mice were allowed to explore the arena and the objects for 10-mins before being removed for a 10-min interval spent in their home cage, located in the test room. Mice were then given a second 10-min sample phase. Two sample phases were used for two reasons, first, preliminary work revealed that two 10-minute sample phases produced robust recognition across delays in WT animals, and secondly, the study was a replication of the O'Doherty (2005) work, which also used two sample phases. Following the second sample phase, the mice were returned to their home cage for either a 10-min or 24-h retention interval. In the test phase, one of the items was replaced with a novel object (see Figure 3.2a). The time mice spent exploring the novel object, and the time spent exploring the two familiar objects was recorded. The location of the objects and the object that was replaced with the novel item was fully counterbalanced both within and between groups. All objects (novel and familiar) and the arena were wiped down with a 5% alcohol/distilled water wipes between sample phases and prior to returning the mouse to the apparatus for the test stage. The order in which mice received the 10-min or 24-h delay was counterbalanced. Discrimination ratios were calculated to index the animals' discriminative performance which was independent of individual differences in contact times. This was calculated as follows: time exploring the novel object/ ((time spent exploring the novel object) + (average time exploring both familiar objects)).

3.2.4.2 Experiment 1b: Tc1 novel object recognition following a 10-min or immediate delay

To determine whether the deficit in short-term recognition memory was confined to a delay of 10-min, we compared the effects of a very short (''immediate") delay between the sample and test trial with a 10-min delay interval. The duration of the ''immediate" delay interval was the time taken to remove the mouse from the arena after the last sample trial, place it in its home cage, and replace one of the objects with a novel object and clean all objects and arena. The average time was approximately 30 sec. The order in which mice received the 10-min, or immediate delay was fully counterbalanced. Discrimination ratios were calculated in the same manner as Experiment 1a.

3.2.4.3 Experiment 2a: Tc1 Object-in-Place memory following a 24-h or immediate delay.

The main aim of this experiment was to further investigate the processing in the MTL of Tc1 mice, by exploring object-place memory in these mice. The two sample phases were identical to those used for the object recognition task. However, in the test phase, two of the objects swapped their spatial locations. This resulted in two familiar objects located in different positions, and one familiar object that remained in its original location (see Figure 3.2bi). The delay period before administering the test was either immediate or 24 h. The rationale for selecting these intervals was that Tc1 mice would potentially be unable to discriminate objects following a 10-min delay (see results of Experiments 1a and 1b) and this would confound assessment of place recognition in Tc1 mice. The objects that exchanged their spatial locations were counterbalanced, and the location of the objects in the arena was also counterbalanced to avoid spatial biases. The order in which mice received the 24 h or immediate delay was counterbalanced. Discrimination ratios were calculated as follows: average time exploring the two objects in different locations/ ((time exploring the object in the same location) + (average time exploring the two objects in different locations)).

3.2.4.4 Experiment 2b: Tc1 novel object location memory following a 10-min delay

The main aim of this experiment was to assess whether Tc1 mice were sensitive to a change in the spatial organisation of objects in the arena that was independent of the ability to discriminate between different objects. Spatial novelty was tested at a ten minute interval. The mice were placed in the centre of the arena and presented with three identical objects, each located in a corner of the square arena. Mice were allowed to explore the arena and the objects for 10-min before being removed and placed back in their home cages for a 10-min interval. Mice were then given a second 10-min sample phase before being placed back in their home cages for a 10-min retention interval prior to a test phase. In the test phase, one of the objects was moved from its original location to the previously vacant corner of the arena (see Figure 3.2bii); all objects and the arena were cleaned prior to the mouse being returned for the test phase. The time mice spent exploring the object in the novel location, and the time spent exploring the two objects in the same location were recorded. The location of the objects in the arena, and the object that was moved to the vacant corner were fully counterbalanced. Discrimination ratios were calculated as follows: time exploring the object in the novel location/ ((time exploring the object in the novel location) + (the average time exploring the two familiar object locations)).

3.2.4.5 Experiment 3: Tc1 novel odour recognition

The main aim of this experiment was to test the generality of the recognition memory deficit in Tc1 mice and thus whether the short-term memory impairment extended to olfactory stimuli. The mice received test trials with visually identical plastic cubes each containing a different scent. Mice were placed in the centre of the arena, and presented with three odour cubes, each in a different corner of the arena. The sample procedure was otherwise identical to Experiment 1a. Following a 10-min or 24-h delay after the last sample trial, the mice received a test trial in which one of the odour cubes was replaced with a novel odour cube (see Figure 3.2c). The test phase was identical to that described for Experiment 1a. The location of the odour cubes in the arena and the odour that was replaced for the test trial were counterbalanced, other aspects of the procedure were identical to Experiment 1a. A discrimination ratio was calculated as in Experiment 1a.

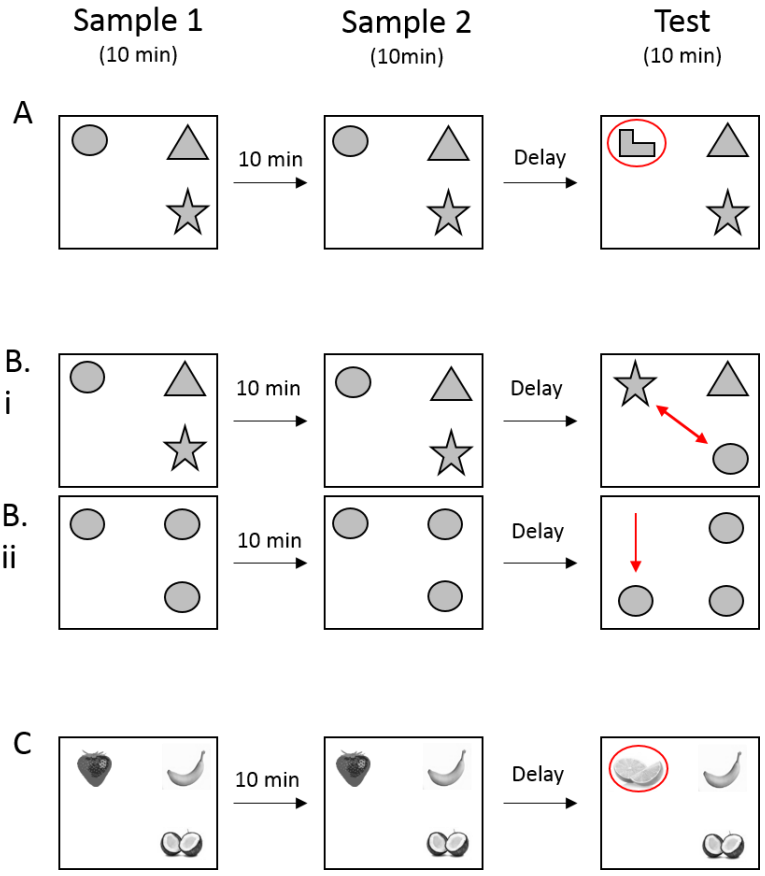


Figure 3.2 – Schematic representations of tasks *(A) Novel object recognition. Mice were exposed to three objects during two 10-min sample phases. After either an immediate (approximately 30 sec), 10-min, or 24-h delay, mice were returned to the arena for the test phase, during which one of the objects was replaced with a novel object. (Bi) Object-in-place. Mice were exposed to three objects during two 10-min sample phases. After a delay of either 24-h, or immediately following the sample stage, mice were returned to the arena for the test phase. During the test, two of the objects swapped their spatial location (see arrow). (Bii) Object location. Mice were exposed to three identical objects during two 10-min sample phases. After a delay of 10-min, mice were returned to the arena for the test phase, during which one of the objects was moved from its original location to a previously vacant corner of the arena. (C) Novel Odour Recognition. Mice were exposed to three visually identical cubes each containing a different odour during two 10-min sample phases. After a delay of either 10-min or 24-h, mice were returned to the arena for the test phase. During the test, one of the odour cubes was replaced with a cube containing a novel odour.*

3.2.4.6 Experiment 4: Tg2576 novel object recognition following a 10- min or 24-h delay

To determine whether the pattern of recognition memory changes in Tc1 mice was specific to expression of Hsa21 and not a non-specific consequence of human gene expression, we examined the performance of aged Tg2576 mice on the same behavioural procedure. Tg2576 mice express a human Swedish APP mutation linked to early onset Alzheimer's disease. A comparison of the performance of Tg2576 mice with Tc1 mice were also of theoretical interest because the overexpression of APP is absent in Tc1 mice, unlike Down syndrome individuals. Therefore it is interesting to explore the impact of APP overexpression on this task. The same novel object recognition protocol described for Experiment 1a was used with one important change. Previous experiments have shown that Tg2576 mice display lower contact times with objects (Hale & Good, 2005). This was confirmed in the present study and indeed one transgenic mouse was removed from the experiment because it consistently failed to make contact with the object during a preliminary assessment of exploratory activity (data not shown; this mouse was excluded from all subsequent data analysis; n = 10 per group). In order to equate exposure times during each sample stage, the exploration times of WT mice were yoked to those shown by Tg2576 mice. This was achieved by pairing WT and Tg2576 mice together for the duration of the experiment. For each pair, the Tg2576 animal was run first on the task and the contact times during each sample stage recorded. The paired WT mouse was then subsequently run, and allowed to accumulate the same contact times with objects as their yoked transgenic mouse. For WT mice, the experimenter stopped each sample exposure once the cumulative total object exploration times matched that of the yoked Tg2576 mouse. Note, there was no attempt to match exploration times with each individual object. The mouse was free to move around the arena. If a WT mouse did not achieve a comparable contact time during the sample phase, the mouse remained in the arena for a maximum of 10 min. During the test phase, WT and Tg2576 mice were given 10-mins to explore the environment freely.

3.2.4.7 Experiment 5: Temporal order memory in Tc1 mice

There are specific components of the MTL circuitry which are required for temporal order memory. Although it must be acknowledged that there is an interactive nature to this system, historically, lesions have demonstrated that ablation of the PFC in rodents leads to an inability to perform temporal order tasks (Barker, Bird, Alexander & Warburton (2007); Hanneson, Howland & Phillips (2003)). In order to interrogate this component of temporal order memory system in the Tc1 mice, this experiment aimed to examine whether recognition memory deficits in the Tc1 mice extended to memory

for temporal order. Mice received test trials with two visually identical objects. In sample phase one, mice were presented with two identical copies of an item. Mice were allowed to explore the arena and the objects for 10-mins before being removed for a one hour interval, spent in their home cage. Mice were then given a second 10-minute sample phase to explore two identical copies of a second item. Following the second sample phase, the mice were returned to their home cage for a 3 hour retention interval (Barker & Warburton, 2011). In the test phase, mice were presented with both a copy of item the first item together with a copy of the second item (figure 3.3). The time mice spent exploring the recently seen object, and the time spent exploring the not recently seen object was recorded. The location of the objects and the order in which animals saw each pair of objects was fully counterbalanced both within and between groups. All objects (novel and familiar) and the arena were wiped down with a 5% alcohol/distilled water wipes between sample phases and prior to returning the mouse to the apparatus for the test stage. Discrimination ratios were calculated as follows: time exploring the not recently seen object/ ((time spent exploring the not recently seen object) + (average time exploring both not recently seen and recently seen objects)).

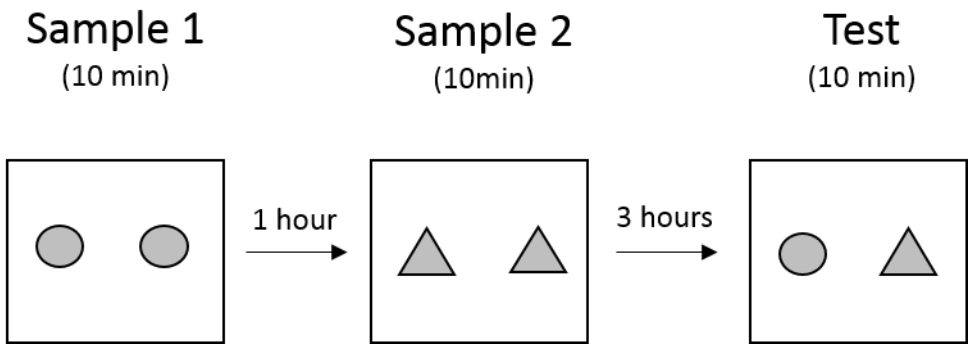


Figure 3.3 – Schematic representation of the temporal order task. *Mice were exposed to two identical objects during the first 10- minute sample phase, then following a one hour delay, mice were exposed to a second pair of identical objects. Following a three hour delay, mice were returned to the arena for the test phase, during which they were presented with one copy of each of the items from sample phase 1 (non-recent) and from sample phase 2 (recent).*

3.2.5 Data analyses

As described in Chapter 2.

3.3 Results

3.3.1 Experiment 1a: Tc1 Novel object recognition following a 10-min or 24-h delay

The main aim of this experiment was to test the hypothesis that Tc1 mice will show impaired short- but intact long-term object recognition memory. The contact times for each group of mice during the sample phases (collapsed across retention interval conditions) are shown in Table 3.1 and contact times during the test phase in Table 3.2, respectively. Inspection of Table 3.1 suggests that Tc1 mice showed numerically higher contact times with the objects than WT mice. However, the duration of contact decreased in both Tc1 and WT at a similar rate across the sample phases. An Analysis of variance (ANOVA) with sample phase as the within subjects factor, and genotype as the between subjects factor revealed a significant main effect of sample phase on object contact time ($F_{(1, 23)} = 18.264$, $p < 0.001$), but no significant main effect of genotype ($F_{(1, 23)} = 3.767$, $p = 0.065$), and no significant interaction between these variables ($F < 1$, $p = 0.971$). This shows that although the Tc1 mice interacted with the objects more than the WT animals, both groups showed a significant decrease in activity (habituation) from sample phase 1 to sample phase 2. Table 3.2 shows the mean contact times with objects (novel and familiar) across the delay conditions for Tc1 and WT mice. A repeated measures ANOVA using object and delay as the within subjects factors, and genotype as the between subjects factor revealed a significant main effect of object ($F_{(1, 23)} = 66.156$, $p < 0.001$) but no significant main effect of delay ($F < 1$, $p = 0.567$), and no significant main effect of genotype ($F < 1$, $p = 0.796$). The interaction between object and genotype, failed to reach statistical significance ($F_{(1, 23)} = 3.356$, $p = 0.080$). There was no significant interaction between object and delay ($F_{(1, 23)} = 1.472$, $p = 0.237$) or three-way interaction between object, delay and genotype ($F < 1$, $p = 0.479$).

In order to evaluate performance that was independent of individual differences in contact times, the data were also analysed using a discrimination ratio and are shown in Figure 3.4. Inspection of this figure indicates that wild type control mice discriminated between novel and familiar objects following both a 10-min and 24-h delay. In contrast, the Tc1 mice successfully discriminated between novel and familiar objects only following the 24-h delay. A repeated measures ANOVA using discrimination ratios as the within subjects factor and genotype as the between subject factor revealed a significant main effect of genotype ($F_{(1, 23)} = 7.076$, $p < 0.05$), but no significant main effect of delay ($F_{(1, 23)} = 1.726$, $p = 0.202$). There was, however, an interaction between these two factors ($F_{(1, 23)} = 6.069$, $p = 0.05$). Tests of simple main effects revealed a significant effect of genotype at the 10-min delay ($F_{(1, 23)} = 11.176$, $p < 0.05$), but not at the 24-h

delay ($F < 1$, $p = 0.736$). Furthermore, one sample t-test confirmed that the performance of the WT mice were significantly above chance at both delays (10 min: $t_{(11)} = 8.03$, $p < 0.001$; 24 h: $t_{(11)} = 4.75$, $p < 0.001$). However, the performance of Tc1 mice was not above chance at the 10 min delay, ($t < 1$), but was above chance at the 24 h delay ($t_{(12)} = 6.57$, $p < 0.001$). These results therefore confirm that Tc1 mice showed impaired short-term but intact long-term object recognition memory.

3.3.2 Experiment 1b: Tc1 Novel object recognition following a 10-min or immediate delay

The main aim of Experiment 1b was to determine whether Tc1 mice would show a recognition memory deficit when tested immediately after the sample phase; considering the deficit at a short term 10- minute interval, the aim was to establish a more specific time frame during which the recognition memory deficit was present. This would establish whether sensory processing or coding was affected in the Tc1 mice. Considering that the Tc1 mice show intact recognition memory at 24-hrs, this would suggest that encoding is intact. Investigating performance at an immediate time point would help to further establish this. The mean contact times shown by WT and Tc1 mice during the sample phases are shown in Table 3.1. An ANOVA, with sample phase as the within subjects factor and genotype as the between subjects factor revealed a significant main effect of sample phase on contact times ($F_{(1, 14)} = 8.351$, $p < 0.05$), but no significant main effect of genotype ($F < 1$, $p = 0.798$), and no significant interaction between these variables ($F < 1$, $p = 0.431$). This result confirmed that both groups interacted with the objects at the same level and habituated to the stimuli across the sample phases to a similar extent. The mean object contact times during the novelty recognition test are shown in Table 3.2. Inspection of this table shows that Tc1 mice displayed a normal object novelty preference when tested immediately, but not following a 10-min delay. A repeated measures ANOVA using object and delay as the within subjects factors, and genotype as the between subjects factor revealed a significant main effect of delay ($F_{(1, 14)} = 69.037$, $p < 0.001$) but no significant main effect of object ($F < 1$, $p = 0.395$) or genotype ($F < 1$, $p = 0.569$). There was no significant interaction between object and genotype ($F < 1$, $p = 0.804$). There was, however, a significant three-way interaction between object, delay and genotype ($F_{(1, 14)} = 5.419$, $p < 0.05$). Tests of simple main effects revealed a significant effect of delay on contact times with the familiar object in the Tc1 mice ($F_{(1, 14)} = 11.569$, $p < 0.05$). Tests of simple main effects also revealed a significant effect of object type in WT mice following both the immediate ($F_{(1, 14)} = 16.075$, $p = <0.001$) and 10-min ($F_{(1, 14)} = 46.144$, $p < 0.001$) conditions. There was also a significant effect of object type for Tc1 mice following the immediate test ($F_{(1, 14)} = 24.380$, $p < 0.001$), but not when the test was conducted after 10-min ($F_{(1, 14)} = 3.912$, $p = 0.068$). An analysis of

the discrimination ratios shown in Figure 3.5 revealed a significant main effect of delay ($F_{(1, 14)} = 5.405$, $p < 0.05$), but no significant main effect of genotype ($F < 1$, $p = 0.401$). There was however an interaction between these two factors ($F_{(1, 14)} = 7.642$, $p < 0.05$) and tests of simple main effects revealed a significant effect of genotype following the 10-min delay ($F_{(1, 14)} = 11.986$, $p < 0.05$) but not following the immediate delay condition, ($F < 1$, $p = 0.397$). One sample t-tests confirmed that the performance of the WT was significantly above chance (0.5) at both delays (10 min: $t_{(7)} = 6.55$, $p < 0.001$; immediate: $t_{(7)} = 2.52$, $p < 0.05$). However, Tc1 mice were above chance in the immediate delay condition only (10 min: $t_{(7)} = 1.88$, $p = 0.100$; immediate: $t_{(7)} = 19.67$, $p < 0.001$). The results of this experiment show that Tc1 mice are able to discriminate novel versus familiar objects following an immediate delay but not when tested after a 10-min interval.

Genotype	Mean contact time during the sample phases (seconds) with all objects for Tc1, Tg2576 and WT control mice	
	Sample Phase 1	Sample Phase 2
Tc1	Experiment 1a: Novel object recognition – 10 min vs 24 h delay	
	18.12	13.70
WT	13.28	8.93
Tc1	Experiment 1b: Novel object recognition – 10 min vs immediate delay	
	30.15	23.12
WT	31.33	20.49
Tc1	Experiment 2a: Object in place – 24 h vs immediate delay	
	23.28	17.31
WT	25.20	15.40
Tc1	Experiment 2b: Object location – 10 min delay	
	26.32	21.32
WT	31.03	22.15
Tc1	Experiment 3: Novel odour recognition – 10 min vs 24 h delay	
	13.23	11.03
WT	13.35	10.34
	Experiment 4: Yoked novel object recognition – 10 min vs 24 h delay in Tg2576 mice	
Tg2576 and WT	12.44	8.87
Tc1	Experiment 5: Temporal order – 3 h delay	
	27.60	20.85
WT	25.73	15.49

Table 3.1 *-Mean contact times (seconds) during the sample phase with novel and familiar objects for Tc1, Tg2576 and WT control mice.*

Genotype	Mean contact time (seconds) during the test phase with novel and familiar objects for Tc1, Tg2576 and WT control mice			
	Experiment 1a: Novel object recognition – 10 min vs 24 h delay			
	Novel Object (10 min)	Novel Object (24 h)	Familiar Object (10 min)	Familiar Object (24 h)
Tc1	5.692	7.132	4.048	2.956
WT	6.399	7.716	2.120	2.791
	Experiment 1b: Novel object recognition – 10 min vs immediate delay			
	Novel Object (10 min)	Novel Object (Immediate)	Familiar Object (10 min)	Familiar Object (Immediate)
Tc1	10.910	16.863	8.647	4.083
WT	12.963	11.790	5.190	3.724
	Experiment 2a: Object in place – 24 h vs immediate delay			
	Novel Place (24 h)	Novel Place (Immediate)	Familiar Place (24 h)	Familiar Place (Immediate)
Tc1	9.323	9.909	3.369	3.959
WT	12.422	7.191	5.378	3.215
	Experiment 2b: Object location – 10 min delay			
	Novel Location (10 min)		Familiar Location (10 min)	
Tc1	13.060		5.894	
WT	19.729		4.616	
	Experiment 3: Novel odour recognition – 10 min vs 24 h delay			
	Novel Odour (10 min)	Novel Odour (24 h)	Familiar Odour (10 min)	Familiar Odour (24 h)
Tc1	8.819	7.413	7.580	3.640
WT	7.493	7.209	2.821	3.240
	Experiment 4: Yoked novel object recognition – 10 min vs 24 h delay in Tg2576 mice			
	Novel Object (10 min)	Novel Object (24 h)	Familiar Object (10 min)	Familiar Object (24 h)
Tg2576	4.774	1.759	1.924	1.767
WT	10.543	13.698	4.499	6.244
	Experiment 5: Temporal order – 3 h delay			
	Non-Recent Object (3 h)		Recent Object (3 h)	
Tc1	10.949		4.804	
WT	11.186		5.576	

Table 3.2 *-Mean contact times (seconds) during the test phase with novel and familiar objects for Tc1, Tg2576 and WT control mice.*

3.3.3 Experiment 2a: Tc1 Object-in-Place memory following a 24-h or immediate delay

The above experiments indicate that Tc1 mice display impaired memory for object information following a short but not long delay interval. The main aim of this experiment was to test the hypothesis that the reported impairment in hippocampal synaptic plasticity and place cell activity in Tc1 mice (O'Doherty et al., 2005; Witton et al., 2015) would disrupt memory for the spatial organisation of objects. The mean contact times with the objects during the sample stages are shown in Table 3.1. Inspection of these data indicate that WT and Tc1 mice showed a comparable reduction in contact times across sample phases. An ANOVA revealed a significant main effect of sample phase on contact time ($F_{(1, 14)} = 18.701$, $p < 0.005$), but no significant effect of genotype ($F < 1$, $p = 0.999$), and no significant interaction involving these factors, ($F_{(1, 14)} = 1.105$, $p = 0.311$). Mean contact times with the objects during the test trial are shown in Table 3.2. WT and Tc1 mice showed comparable exploration of the novel object-location pairings both immediately and 24-h following the last sample trial. A repeated measures ANOVA using object and delay as the within subjects factors, and genotype as the between subjects factor revealed a significant main effect of object ($F_{(1, 14)} = 103.726$, $p < 0.001$), but no significant main effect of delay ($F_{(1, 14)} = 2.701$, $p = 0.123$) or genotype ($F < 1$, $p = 0.801$).There was a significant delay x genotype interaction ($F_{(1, 14)} = 4.740$, $p < 0.05$). Simple main effects revealed no significant effect of genotype at either the immediate ($F_{(1, 14)} = 1.07$, $p = 0.318$) or 24-h condition ($F_{(1, 14)} = 3.878$, $p = 0.069$). The main effect of delay was not significant for Tc1 mice ($F < 1$, $p = 0.712$) but was for WT mice ($F_{(1, 14)} = 7.298$, $p < 0.05$). There was no significant three-way interaction between object, delay and genotype ($F_{(1, 14)} = 3.39$, $p = 0.087$). An analysis of the discrimination ratio data (see Figure 3.6) demonstrated a similar pattern. An ANOVA showed no main effect of genotype ($F < 1$, $p = 0.971$), or delay ($F < 1$, $p = 0.735$), and no significant genotype x delay interaction ($F < 1$, $p = 0.408$). One sample t-tests confirmed that the performance of both the WT (24 h: $t_{(7)} = 6.08$, $p < 0.001$; immediate: $t_{(7)} = 4.88$, $p < 0.001$) and Tc1 mice (24 h: $t_{(7)} = 8.77$, $p < 0.001$; immediate: $t_{(7)} = 4.14$, $p < 0.01$) were significantly above chance (0.5) at both delays. These results indicate that despite evidence for impaired hippocampal synaptic plasticity and place cell activity in Tc1 (Witton et al., 2015), these animals remained sensitive to a mismatch in object-location information following an immediate or 24-h delay.

3.3.4 Experiment 2b: Tc1 Novel object location memory following a 10-min delay

In the previous experiments Tc1 mice showed impaired novelty detection following a 10-min delay. As a result, the object-in-place task could not be carried out at a 10-min delay, as it relies not only on spatial recognition memory, but also relies on the encoding of object information. Therefore, it is not possible to draw conclusions about the spatial recognition memory abilities of the Tc1 mice at a 10-min delay. The main aim of the present experiment therefore was to determine whether Tc1 mice were able to react to spatial novelty on a novel location task, whereby a familiar object was moved to a completely novel location using a 10-min delay interval. This task however, unlike the object-in-place task, does not rely upon the ability to discriminate between objects, as the objects are identical. This task is not sensitive to bilateral lesions of the perirhinal cortex but is impaired following hippocampal damage (Barker & Warburton, 2011). Considering the aberrant hippocampal short-term, but not long-term, synaptic plasticity, abnormal hippocampal spine morphology, and sub-region changes in the connectivity of the DG-CA3 network that contributes to disruption of place-cell activity (O'Doherty et al., 2005; Witton et al., 2015). This would suggest that memory for the spatial organisation of objects will be disrupted in Tc1 mice, thus it is hypothesised that Tc1 mice will show a deficit on the object-location task following a 10-min delay.

The mean contact times for WT and Tc1 mice during the sample stages are shown in Table 3.1. An ANOVA revealed a significant main effect of sample phase on object exploration ($F_{(1, 14)} = 9.009$, $p < 0.05$), but no significant effect of genotype ($F < 1$, $p = 0.491$), and no significant genotype x sample phase interaction, ($F < 1$, $p = 0.416$). The mean contact times for both groups during the object location test are shown in Table 3.2. Inspection of these data show that both WT and Tc1 mice remained sensitive to the movement of a familiar object to a novel location following a 10-min retention interval. An ANOVA revealed a significant main effect of object ($F_{(1, 14)} = 5.597$, $p < 0.05$), but no significant effect of genotype ($F < 1$, $p = 0.581$), and no significant object x genotype interaction ($F_{(1, 14)} = 3.428$, $p = 0.085$). An analysis of the discrimination ratios (see Figure 3.7) confirmed the performance of the WT and Tc1 mice was comparable ($t_{(14)} = 1.169$, $p = 0.262$) and that both WT ($t_{(7)} = 4.4$, $p < 0.01$), and Tc1 mice ($t_{(7)} = 2.55$, $p < 0.05$), performed above chance. With reference to experiments 1a and b, the results of the present experiment are important because they show that Tc1 mice were able to process and react to novelty and specifically spatial novelty following a 10-min retention interval. This indicates that the object novelty impairment of Tc1 mice at the 10 min delay is not a reflection of a deficit in either detecting novelty or modifying exploratory behaviour following a 10-min retention interval.

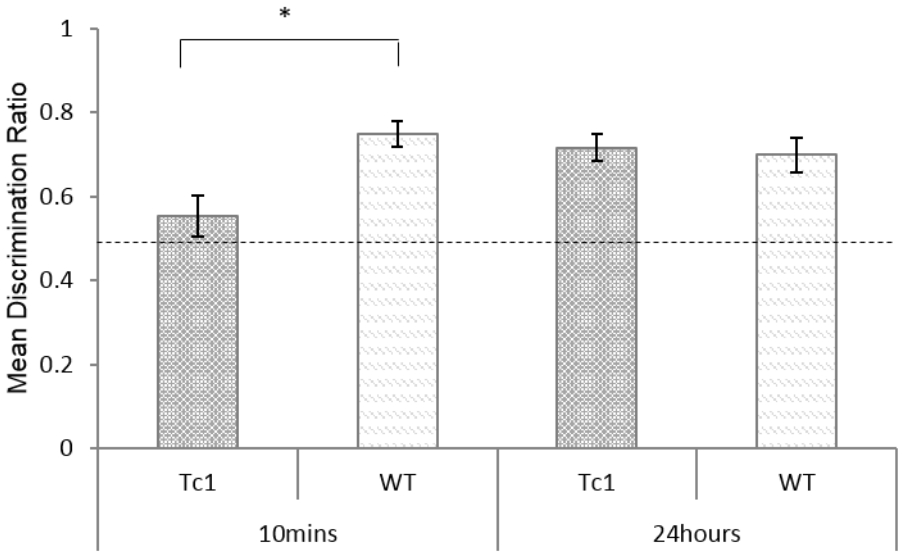


Figure 3.4- Results of experiment 1a: *Novel object recognition following a 10-min or 24-h delay in Tc1 and WT control mice. Mean discrimination ratios (error bars represent ± SEM) describing the preference for the novel object for Tc1 and wild type (WT) mice (* = p < 0.05, --------- = chance level (0.5)).*

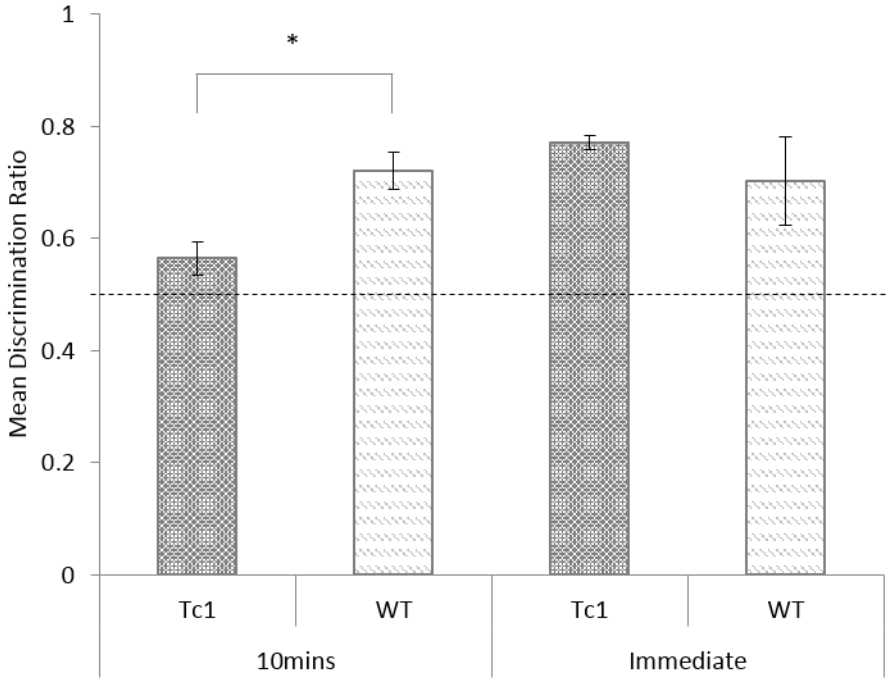


Figure 3.5 – Results of experiment 1b*: Novel object recognition memory following an immediate or 10 min delay interval in Tc1 and WT mice. Mean discrimination ratios (error bars show ± SEM) for Tc1 and WT mice showing the preference for the novel object following either an immediate or 10-min retention interval (* = p < 0.05, --------- = chance level (0.5)).*

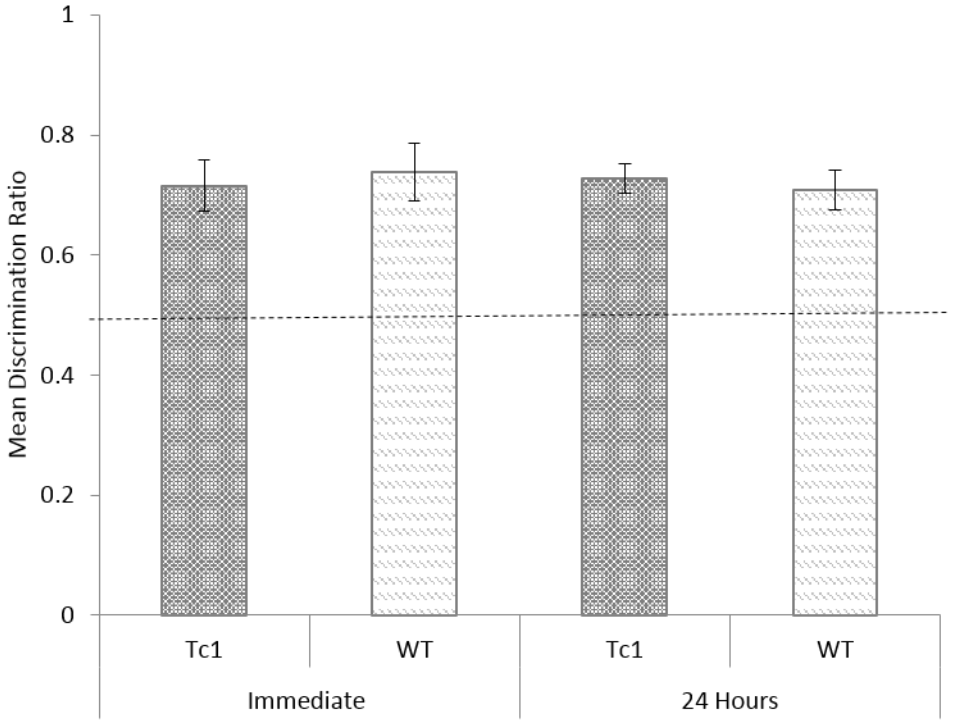


***Figure 3.6 – Results of experiment 2a**: Object-in place memory in Tc1 and WT control mice following an immediate or 24-h delay. Discrimination ratio (error bars represent the ± SEM) describing the preference for the objects in a different (but familiar) location following either an immediate or 24-h delay for Tc1 and WT control mice. (--------- = chance level (0.5)).*

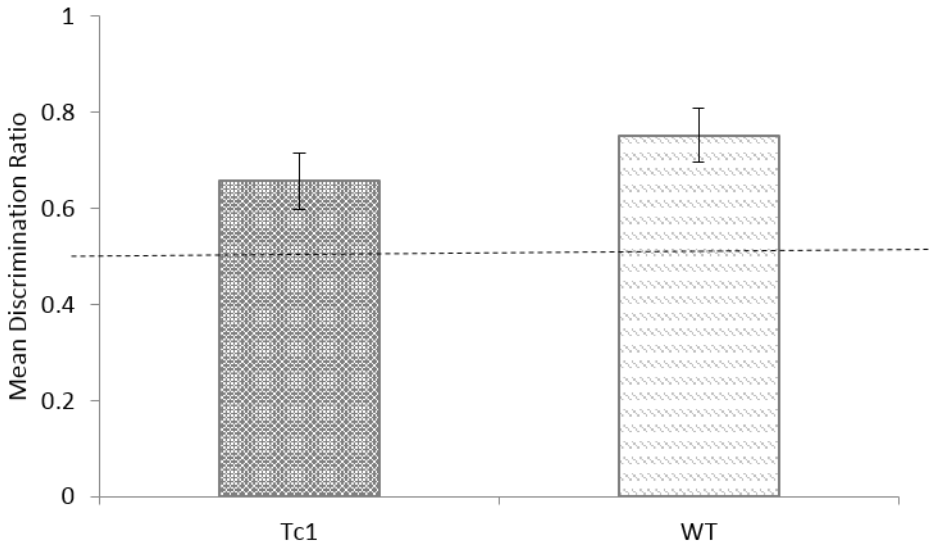


***Figure 3.7 – Results of experiment 2b**: Object location memory following a 10-min delay in Tc1 and WT control mice. Discrimination ratio (error bars ± SEM) describing the preference for the object moved to a completely novel location in the arena. (--------- = chance level (0.5)).*

3.3.5 Experiment 3: Tc1 Novel odour recognition

The aim of this experiment was to test the hypothesis that the Tc1 impairment in short-term recognition memory was not sensory domain specific in order to ensure that any deficit on object recognition memory tasks are not simply due to a problem with the function of the visual cortex. The perirhinal cortex also processes olfactory information (Otto and Eichenbaum, 1992), and so exploring the odour recognition abilities in the Tc1 mouse will establish whether the problem transcends more than one sensory domain. The contact times for WT and Tc1 mice during the odour sample stages are shown in Table 3.1. An ANOVA revealed a significant main effect of sample phase on contact times ($F_{(1, 22)} = 15.025$, $p < 0.001$), but no significant main effect of genotype ($F < 1$, $p = 0.790$). There was no significant interaction between these variables ($F < 1$, $p = 0.549$). This analysis confirmed that both groups showed contact time habituation across the sample phases. The mean contact times for the olfactory novelty test are shown in Table 3.2. Inspection of this table shows that Tc1 mice, unlike WT mice, showed a weaker preference for the novel odour following the 10-min delay relative to the 24-h delay. An ANOVA revealed a significant main effect of odour ($F_{(1, 22)} = 86.624$, $p < 0.001$), a significant effect of delay ($F_{(1, 22)} = 7.657$, $p < 0.001$), and a significant main effect of genotype ($F_{(1, 22)} = 4.803$, $p < 0.05$). There was also a significant delay x genotype interaction ($F_{(1, 22)} = 8.473$, $p < 0.05$). Tests of simple main effects revealed a significant effect of genotype at the 10-min delay ($F_{(1, 22)} = 10.492$, $p < 0.05$), but not at the 24-h delay ($F < 1$, $p = 0.728$). There was also a significant genotype x odour interaction ($F_{(1, 22)} = 6.091$, $p < 0.05$). Tests of simple main effects revealed a significant effect of genotype on contact with the familiar odour ($F_{(1, 22)} = 11.606$, $p < 0.05$), but not with the novel odour ($F < 1$, $p = 0.408$). There was also a significant three-way interaction between odour, delay and genotype ($F_{(1, 22)} = 6.979$, $p < 0.05$). Tests of simple main effects revealed a significant effect of odour type on the contact times of WT animals, in both the 10-min ($F_{(1, 22)} = 46.708$, $p < 0.001$), and the 24-h delay ($F_{(1, 22)} = 35.215$, $p < 0.001$) conditions. There was also a significant effect of odour on the contact times of Tc1 mice in the 24-h condition ($F_{(1, 22)} = 31.910$, $p < 0.001$), but not in the 10-min delay condition ($F_{(1, 22)} = 3.292$, $p = 0.083$). An ANOVA carried out on the discrimination ratio data (see Figure 3.8) revealed a similar pattern and showed a significant main effect of genotype ($F_{(1, 22)} = 25.992$, $p < 0.001$), but no significant main effect of delay ($F_{(1, 22)} = 3.103$, $p = 0.092$) and a significant interaction between these two factors ($F_{(1, 22)} = 16.228$, $p < 0.001$). Tests of simple main effects revealed a significant effect of genotype at the 10-min delay ($F_{(1, 22)} = 25.992$, $p < 0.001$), but not on the 24-h delay (F<, $p = 0.979$). One sample t-tests confirmed that the performance of the WT mice was above chance at both delays (10 min: $t_{(11)}$ 8.29, $p < 0.001$; 24-h: $t_{(11)} = 7.52$, $p < 0.001$). However, the

performance of Tc1 mice was above chance only at the 24-h delay ($t_{(11)} = 8.61$, $p < 0.001$) and not following the 10 min delay ($t_{(11)} = 1.93$, $p = 0.070$). These results confirm that Tc1 mice showed impaired short-term but intact long-term recognition memory for odour stimuli.

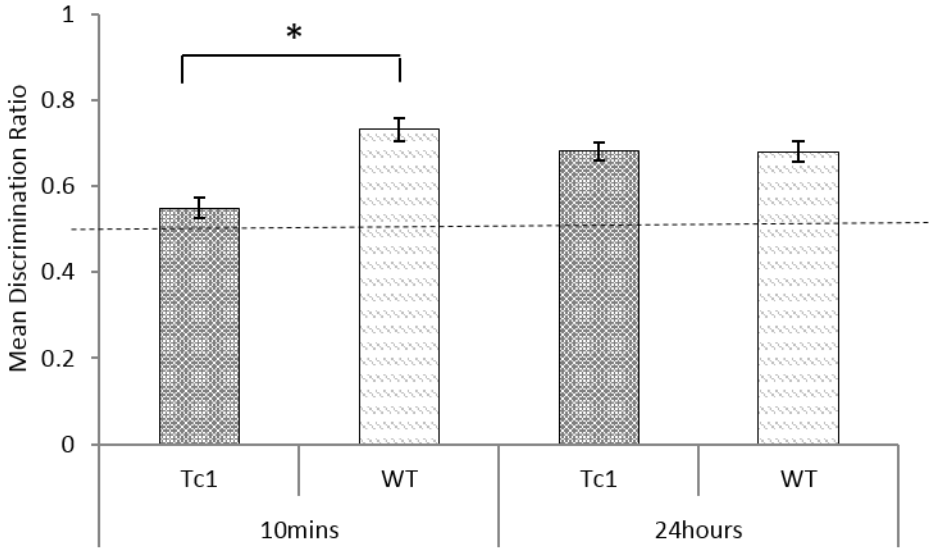


***Figure 3.8 – Results of experiment 3**: Novel odour recognition memory following a 10-min or 24-h delay in Tc1 and WT control mice. Mean discrimination ratios (error bars represent ± SEM) describing the preference for the novel odour following a 10-min or 24-h delay for Tc1 and WT control mice (* = p < 0.05, --------- = chance level (0.5)).*

3.3.6 Experiment 4: Tg2576 Novel object recognition following a 10- min or 24-h delay

The main aim of Experiment 4 was to determine whether the pattern of impaired short but intact long-term object recognition memory was specific to the Tc1 mouse line or a non-specific effect of the expression of human genes on performance. Similarities between the neuropathology in older adults with DS and patients with AD led to work which identified brain Aβ deposits, found in neuritic plaque formations, as being homologous between the two conditions (Glenner and Wong, 1984). The amyloid precursor protein (APP) gene (Selkoe et al. 1988), the product of which is cleaved to yield Aβ peptides has been genetically mapped to Hsa21 (Tanzi et al. 1988) therefore confirming APP as a strong candidate for the pathogenesis of AD in DS. It has since been shown that rare duplications of the APP locus alone is sufficient to cause dementia (Rovelet-Lecrux et al. 2006). Two partial trisomy cases in which APP is not duplicated also do not present with dementia, implying that APP is necessary as well as sufficient to trigger the AD phenotype (Prasher et al. 1998, Korbel et al. 2009). The overexpression of APP however is absent in Tc1 mice, unlike Down syndrome individuals, and a comparison with a mouse model which does overexpress APP would be of interest. Tg2576 mice express the ‘‘Swedish” amyloid precursor protein mutation (HuAPP695SWE; driven by a hamster prion protein promoter); (Hsiao et al., 1996). We therefore examined the effects of the overexpression of a mutant human APP mutation, linked to an early-onset form of Alzheimer’s disease, in the Tg2576 mice on object recognition memory.

The contact times during the sample phases for WT and Tg2576 mice were yoked, and the mean contact times are shown in Table 3.1. An ANOVA confirmed that the two groups were matched for contact times during the sample phases, with no significant main effect of genotype ($F < 1$, $p = 0.672$). There was a significant main effect of sample phase on contact times ($F_{(1, 18)} = 5.636$, $p < 0.05$) and no interaction between these factors ($F < 1$, $p = 0.534$). The results confirm that both groups showed a significant decrease in contact times (habituation) across the sample phases. The mean contact times for both Tg2576 and WT mice during the test phase of the object novelty task are shown in Table 3.2. A repeated measures ANOVA revealed no significant main effect of delay, ($F < 1$, $p = 0.565$), but a significant main effect of object ($F_{(1, 18)} = 79.941$, $p < 0.001$), and a significant main effect of genotype, ($F_{(1, 18)} = 81.300$, $p < 0.001$). There was no significant delay x object interaction ($F < 1$, $p = 0.454$). There was, however, a significant delay x genotype interaction ($F_{(1, 18)} = 7.493$, $p < 0.05$). Subsequent tests of simple effects revealed a significant effect of genotype at both the 10-min ($F_{(1, 18)} = 103.274$, $p < 0.001$) and the 24-h delays ($F_{(1, 18)} = 20.686$, $p< 0.001$); reflecting the overall

lower contact times of Tg2576 mice. There was also a significant object x genotype interaction ($F_{(1, 18)} = 33.988$, $p < 0.001$). Simple effects revealed an effect of object for both transgenic ($F_{(1, 18)} = 103.274$, $p < 0.001$) and WT mice ($F_{(1, 18)} = 20.686$, $p < 0.001$). There was a three-way interaction of delay x object x genotype ($F_{(1, 18)} = 5.093$, $p < 0.05$). Simple effects revealed a significant effect of genotype on contact times with the novel object at 10-min ($F_{(1, 18)} = 19.248$, $p < 0.001$), and the familiar object at 10-min ($F_{(1, 18)} = 16.665$, $p < 0.05$). There was also a significant effect of genotype on contact times with the novel object at 24-h ($F_{(1, 18)} = 60.915$, $p < 0.001$) and the familiar object at 24 h ($F_{(1, 18)} = 15.171$, $p = < 0.05$). However, there was no significant effect of delay on contact times with the novel object in Tg2576 mice ($F_{(1, 18)} = 3.564$, $p = 0.075$), or the familiar object ($F < 1$, $p = 0.830$). There was no significant effect of delay on contact times with the novel object in WT mice ($F_{(1, 18)} = 3.902$, $p = 0.064$), but there was a significant effect with the familiar object ($F_{(1, 18)} = 5.892$, $p < 0.05$). There was a significant effect of object type for Tg2576 animals at the 10-min delay ($F_{(1, 18)} = 11.548$, $p < 0.05$), but, importantly, not at the 24-h delay ($F < 1$, $p = 0.994$). There was a significant effect of object type for WT animals following the 10-min delay ($F_{(1, 18)} = 51.924$, $p < 0.001$), and the 24-h delay ($F_{(1, 18)} = 54.188$, $p < 0.001$). The discrimination ratio data are shown in Figure 3.9. Inspection of this figure shows that Tg2576 mice discriminated between novel and familiar objects following a 10-min delay but not following a 24-h delay. An ANOVA revealed no significant main effect of genotype, ($F_{(1, 18)} = 2.863$, $p = 0.108$), but a significant main effect of delay, ($F_{(1, 18)} = 9.903$, $p < 0.05$), and a significant genotype x delay interaction, ($F_{(1, 18)} = 9.060$, $p < 0.05$). Tests of simple effects revealed no effect of genotype at the 10-min delay ($F < 1$, $p = 0.667$), but a significant effect of genotype at the 24-h delay ($F_{(1, 18)} = 6.257$, $p < 0.05$). One sample t-tests confirmed that the performance of the WT mice was above chance at both delays (10 min: $t_{(10)} = 7.66$, $p < 0.001$; 24-h: $t_{(10)} = 5.90$, $p < 0.001$). In contrast, the performance of Tg2576 mice was above chance only at the 10 min retention interval (10-min: $t_{(10)} = 5.93$, $p < 0.001$; 24-h: $t < 1$, $p = 0.93$). This pattern was opposite that shown by Tc1 mice and suggests that the recognition deficit in this model was unlikely to be a non-specific consequence of the expression of human genes. Further implications of these findings for the role of APP trisomy in DS cognitive impairment will be considered in the chapter discussion.

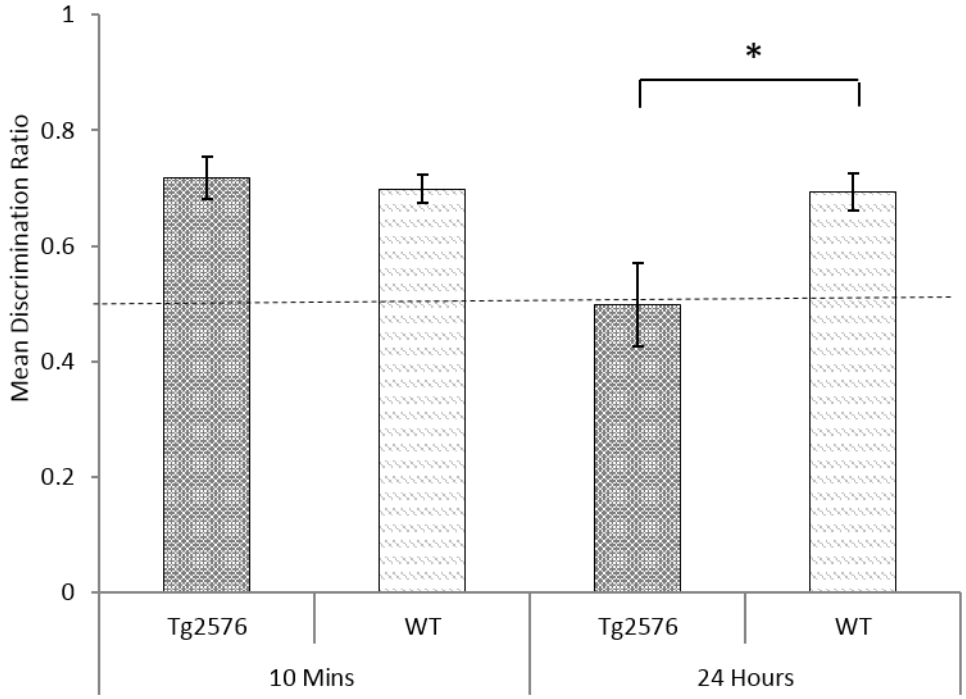


Figure 3.9 –Results of experiment 4*: Novel object recognition following a 10-min or 24-h retention interval in Tg2576 and WT control mice. Mean discrimination ratios (error bars represent the ± SEM) describing the preference for the novel object for Tg2576 and WT control mice following either a 10 min or 24-h delay (* = p < 0.05, --------- = chance level (0.5)).*

3.3.7 Experiment 5: Temporal order memory in Tc1 mice

The main aim of Experiment 5 was to determine whether the pattern of impaired recognition memory extended to temporal order memory in the Tc1 mouse, in order to examine the integrity of the mPFC. Much research has demonstrated difficulty with frontally dependent tasks in DS individuals. For example, Lanfranchi, Jerman, Dal Pont, Alberti and Vianello (2010) carried out a battery of tests of executive function in 15 DS adolescents and 15 mentally age matched controls. The results revealed that the group of individuals with DS performed at a significantly lower level on each of the tasks for set-shifting, working memory and inhibition, suggesting pre-frontal deficits in DS. The medial PFC, like the perirhinal cortex, has also been shown to play an important role in recency discriminations for objects or spatial locations (Barker, Bird, Alexander, & Warburton, 2007). A study by Hannesson, Howland, and Phillips (2004) used bilateral microinfusions of lidocaine into either the anterior perirhinal or medial prefrontal cortex of rats, to block sodium channels immediately before memory testing. This was carried out prior to a familiarity discrimination task and a recency discrimination task. Infusions into the perirhinal cortex disrupted performance in both tasks, whereas infusion into medial PFC only disrupted the recency task performance; the capability of the rats to perform the familiarity discrimination task remained intact. In a second experiment it was found that crossed unilateral inactivation of both structures produced a significant impairment, whereas inactivation of either structure alone produced little or no impairment. This is suggestive of a contribution of the medial PFC to retrieval of long-term object temporal order memory. Given the consistent PFC related deficits seen in DS individuals, then it would follow that DS individuals may have problems with memory for temporal order.

The contact times during the sample phases for WT and Tc1 mice are shown in Table 3.1. An ANOVA confirmed that the two groups were matched for contact times during the sample phases, with no significant main effect of genotype ($F = 0.773$, $p = 0.389$). There was a significant main effect of sample phase on contact times ($F_{(1, 21)} = 7.564$, $p = 0.012$) and no interaction between these factors ($F_{(1, 21)} = 0.318$, $p = 0.579$). The results confirm that both groups showed a significant decrease in contact times (habituation) across the sample phases. The mean contact times for both Tc1 and WT mice during the test phase of the temporal order task are shown in Table 3.2. An ANOVA revealed a significant main effect of object (recent/non-recent) during the test phase ($F_{(1, 21)} = 12.143$, $p<0.001$), but no significant main effect of genotype ($F <1$, $p = 0.765$). There was no significant interaction between these factors ($F <1$, $p = 0.795$). The discrimination ratio data are shown in Figure 3.10. Inspection of this figure shows that Tc1 mice

discriminated between recent and non-recent objects following a 3 hour delay. An analysis of the discrimination ratios confirmed the performance of the WT and Tc1 mice was comparable ($t_{(21)} = 0.996$, $p = 0.331$) and that both Tc1 ($t_{(10)} = 25.0424$, $p < 0.001$), and WT mice ($t_{(11)} = 15.567$, $p < 0.001$), performed above chance.

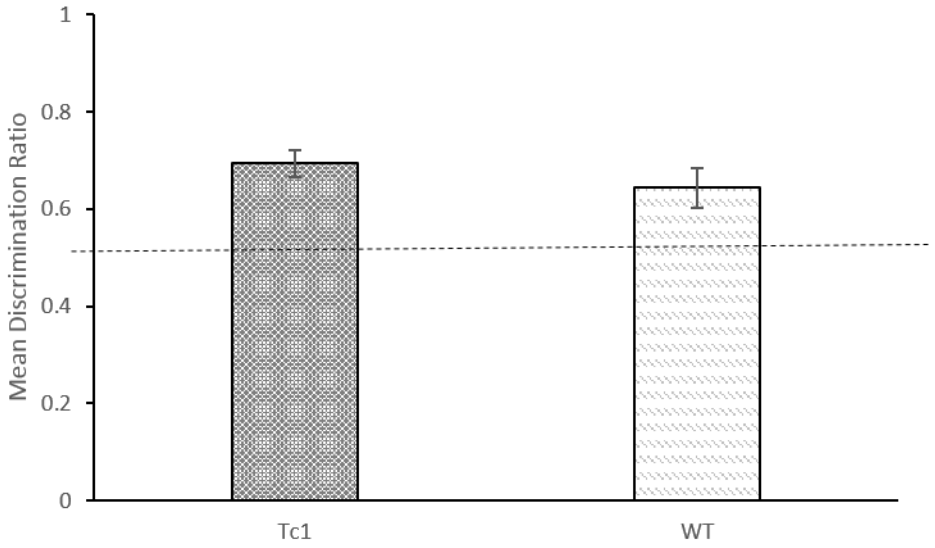


***Figure 3.10 – Results of experiment 5**: Temporal order memory following a three hour retention interval in Tc1 and WT control mice. Mean discrimination ratios (error bars represent the ± SEM) describing the preference for the non-recent object for Tc1 and WT control mice (--------- = chance level (0.5)).*

3.4 Discussion

Previous experiments with Tc1 mice have revealed impaired short-term recognition memory, impaired spatial working memory together with impaired LTP induction, altered hippocampal ultrastructure and impaired place cell activity (Morice et al., 2008; O'Doherty et al., 2005; Witton et al., 2015). The present study supported previous findings, demonstrating a short term impairment in recognition memory in Tc1 mice, despite long term recognition memory remaining intact. The current study also established that memory for object novelty was intact when tested immediately after exposure and that the deficit in short-term object recognition memory extended to olfactory stimuli, demonstrating generalizability of the deficit across sensory domains. In contrast to object memory however, Tc1 mice did not show impairment in tasks of memory for object-place associations when tested either immediately or following a 24 hour delay. Furthermore, Tc1 mice showed normal place recognition following a 10-min delay using an object location task that minimised the necessity for object discrimination, and additionally, showed intact performance on tasks of temporal order memory.

The fact that Tc1 mice were able to perform object location discriminations despite an inability to perform object discriminations has two major implications. Firstly, the data demonstrate that the Tc1 deficit in object and odour recognition memory following a 10 -min delay is specific to an ability to discriminate between novel and familiar objects and odours, and was not a result of a general performance deficit at a 10- minute interval, such as changes in basic locomotor activity, or by dishabituation. It also provides strength to the argument that there is not a general failure to modify exploratory activity following a 10-min retention interval. In addition, although the presence of the Hsa21 gene expression in Tc1 mice disrupted short-term object memory, it failed to impair processing of the spatial attributes of objects at intervals up to 24-hrs, and failed to disrupt memory for temporal order following an interval of three hours, further highlighting the specificity of the behavioural deficit seen in these animals. Finally, the alteration in short-term object memory was specific to Tc1 mice, as Tg2576 mice, that overexpress a human APP mutation, displayed the opposite memory profile to Tc1 mice, demonstrating intact short-term memory but impaired long-term object memory. It is also worth noting that results from the Tg2576 mice are consistent with previous reports that showed normal object novelty detection over short delays using a different object recognition procedure (Good & Hale, 2007). Furthermore, the observation that aged Tg2576 are impaired following a longer, 24 hour interval is consistent with more recent findings, (Oules et al., 2012; Puri, Wang, Vardigan, Kuduk, & Uslaner, 2015). This double dissociation pattern of memory in the different mouse models further emphasises the

genetic specificity of the deficit, and demonstrates that this deficit is not a pattern seen in all rodent models.

In addition, it is important to note that unlike Down syndrome individuals, the overexpression of APP is absent in Tc1 mice. APP plays a major role in brain development and neurogenesis and APP trisomy may contribute to abnormal brain development and cognition (Cataldo et al., 2003, Giacomini et al., 2015 and Trazzi et al., 2013). In this context, it is interesting that Tg2576 mice show intact short term recognition memory, and a deficit in long term recognition memory, a pattern similar to that shown by Ts65Dn mice, and a pattern opposite to that shown by the Tc1 mice (Good & Hale, 2007). This is suggestive of aberrant APP expression, which perhaps alone or in combination with other genes, may contribute to impaired long-term object recognition memory deficits and that other genes may be having an impact on short-term recognition memory processes. Further behavioural assessment of mouse models trisomic for different regions orthologous to Hsa21 will help to address this question.Tg2576 mice express a human Swedish APP mutation linked to early onset Alzheimer's disease.

There is a substantial amount of evidence that the perirhinal cortex plays a key role in processing object identity (Bussey & Saksida, 2005) and object familiarity/novelty discriminations (Brown & Xiang, 1998). Lesions of the perirhinal cortex have been shown to impair memory for objects (Norman & Eacott, 2004). In contrast, bilateral lesions of the perirhinal cortex fail to impair performance on object-location tasks, where the objects are identical and the task relies on spatial representations, and there is no necessity for object discrimination *per se* (Barker & Warburton, 2011; Barker, Bird, Alexander, & Warburton, 2007). The contribution of the hippocampus to object novelty remains controversial with some studies reporting deficits in object recognition memory (Clark, Zola, & Squire, 2000; Hammond et al., 2004) and others reporting no impairment (Warburton & Brown, 2015). The robust deficit of Tc1 mice in short-term but not long-term object recognition memory suggests that any hippocampal or perirhinal cortex contribution to long-term object recognition memory remained intact.

Recent work has illustrated the importance of both the hippocampus and frontal cortex in recognition memory processes. For example, disconnection studies have shown that the hippocampus functions as part of an integrated network with the perirhinal cortex and medial prefrontal cortex supporting object-in-place memory but not object-location memory (Barker et al., 2007; Barker & Warburton, 2011). The medial prefrontal cortex contributes to temporal order judgements and functionally interacts with the hippocampus and perirhinal cortex to form object-in-place associations (Barker et al., 2007). Disconnection studies have also demonstrated that a functional connection between the perirhinal cortex and the hippocampus was not required for object

recognition memory but was necessary for successful object-in-place memory and object recency memory (Barker & Warburton, 2011; Jo & Lee, 2010). Thus, there is an integrated network of structures that contributes to identity, spatial and temporal properties of recognition memory in rodents.

Tc1 mice consistently showed deficits in object recognition memory following a 10-minute delay but not immediately or following 24-hrs. One interpretation of this is that short-term memory processes in the perirhinal cortex are impaired by the mutation. Additionally however, a study by Witton et al. (2015) recently reported that Tc1 mice showed abnormal hippocampal place cell activity, hippocampal synaptic morphology and impaired spatial radial-arm working memory. The presence of hippocampal synaptic and place cell deficits suggests that the contribution of this structure to place recognition memory in Tc1 mice should be impaired. The present results clearly contradict this view. Although hippocampal abnormalities in Tc1 mice appear to be sufficient to transiently impair spatial working memory (Witton et al., 2015), they are not sufficient to disrupt processing or memory for the visuo-spatial properties of object arrays using the described current testing procedures and parameters.

It remains possible, of course, that object-in-place and location memory may be disrupted in Tc1 mice under conditions that place greater demand on memory resources. For example, by increasing the number of object locations or the spatial similarity between object locations. For example, Smith, Kesner, and Korenberg (2014) carried out a task of metric spatial processing in Ts65Dn mice, whereby during the sample phases, mice were exposed to two objects spaced 25 cm apart. During the test phase, identical copies of the same objects were moved closer towards each other so that the objects were now 8 cm apart. Re-exploration during the test phase was used as an index of detecting a metric change. Ts65Dn demonstrate a deficit on this task. In this task, the sample phase and the test phase share more overlapping features than a standard object-in-place protocol, which may lead to increased difficulty with pattern separation. Pattern separation is a computational process that has long been associated with the dentate gyrus, and it occurs when the output firing patterns of a network are less similar to one another than the input firing patterns (Clelland et al., 2009). The differences in firing patterns can occur either through rate modulation of neurons within a population or by the firing of a unique set of neurons to each input (Deng, Aimone, & Gage, 2010). If two events are encoded too similarly, the stored memories may converge into a single memory, rendering future pattern completion, and thereby recall, impossible. The introduction of overlapping representations increases the possibility of interference as a result of similar inputs. This problem is more likely to occur if the memories have an increased number of overlapping features. Thus, Tc1 mice may display a deficit with

spatial memory tasks that place more demand on the pattern separation function of the dentate gyrus; particularly considering that Tc1 mice do show reduced LTP, specifically in the DG, and impaired short-term plasticity at dentate gyrus–CA3 excitatory synapses (Witton et al., 2015). Further work would be required to clarify this.

Perhaps one of the most interesting aspects of the present pattern of results is that short-term and long-term recognition processes were dissociated in Tc1 mice. One interpretation of this finding is that in Tc1 mice, cortical systems supporting short-term object memory were disrupted. Although the delay boundary remains to be established, nevertheless, similar dissociations between short- and long-term recognition memory have been reported following manipulation of kainate or cholinergic receptors in the perirhinal cortex. Barker et al. (2006) reported that infusion of a kainate receptor antagonist UBP302 (a selective GLUK5 antagonist) into the perirhinal cortex impaired recognition memory following a short (20-min) delay but not following a long (24-h) delay. Antagonism of perirhinal NMDA receptors produced the opposite pattern of results. In other work, Tinsley et al. (2011) showed that antagonism of muscarinic cholinergic receptors in the perirhinal cortex impaired short (20-min), but not long-term (24-h) recognition memory. These results argue for distinct and independent short and long-term memory processes in the perirhinal cortex (Barker et al., 2006). It remains possible that trisomy of Hsa21 genes in Tc1 mice may impact on these cortical receptors. There is evidence for polymorphisms in GluK1 kainate receptors in DS (Ghosh, Sinha, Chatterjee, & Nandagopal, 2009), as well as evidence for decreased microtubule motor protein KIF17 expression in trisomic mice, which may alter the distribution of GluK1 localization in distal dendrites (Kayadjanian, Lee, Pina-Crespo, & Heinemann, 2007; Roberson, Toso, Abebe, & Spong, 2008). It remains possible that changes in kainate receptor activity in the perirhinal cortex could underpin the disruption of short-term recognition memory in Tc1 mice. Although the cholinergic projections to the perirhinal cortex in Tc1 mice have not been characterised, to our knowledge, other DS mouse models, such as Ts65Dn, display age-related changes in the cholinergic system (Ash et al., 2014; Granholm et al., 2002; Kelley et al., 2014). It remains possible that either cholinergic innervation of the perirhinal cortex or expression/activity of perirhinal kainite receptors is altered in Tc1 mice. Further work regarding this hypothesis will be discussed in chapter 5.

The pattern of recognition memory deficits displayed by Tc1 mice differ significantly from that shown by Ts65Dn mice, one of the most commonly used models of DS. Ts65Dn mice are a segmental trisomy model of DS and are trisomic for approximately 56% of genes on mouse chromosome 16 that are homologues for human chromosome 21 (Ruparelia, Pearn, & Mobley, 2013). One important difference between the two models

is that the amyloid precursor protein (APP) is not trisomic in Tc1 mice, whereas it is present in three copies in Ts65Dn mice (Choong, Tosh, Pulford and Fisher, 2015). Further discussion of the differences between the Tc1 mouse, and the Ts65Dn and Tg2576 with relation to the patterns of recognition memory they each show will be reserved for the general discussion.

The alteration in short-term memory in Tc1 mice is broadly consistent with some changes in memory that are observed in individuals with DS. Impairments in verbal and non-verbal object memory are commonly reported in DS (Vicari, Bellucci, & Carlesimo, 2005; Vicari, Bellucci, & Carlesimo, 2006; Vicari, Carlesimo, & Caltagirone, 1995). Furthermore, processing of visuo-spatial information is relatively spared in DS (Edgin, Pennington, & Mervis, 2010); although recent evidence indicates impaired allocentric memory in DS children, consistent with disruption of the hippocampus and related cortical regions (Lavenex et al., 2015). In this study, children with DS were asked to find hidden rewards located under cups in an open-field arena and their performance on this allocentric navigation task was contrasted with a condition in which the reward cups were identified by a unique feature. Children with DS were impaired on the allocentric navigation version relative to the cued condition. It would be of interest to compare the mice on a similar procedure to that used by Lavenex et al. (2015) and establish whether the presence of cued trials differentially overshadowed learning of the spatial location of rewards in Tc1 mice. Although the present study has shown that Tc1 mice are able to acquire and retain multiple object-in-place associations, it would be of interest to determine if Tc1 mice perform as well as well as WT mice on a navigation task in which cued information is necessary for navigation, as opposed to relying on just egocentric spatial navigation tactics.

Tasks which have investigated the role of the mPFC in temporal order memory in rodents have demonstrated that bilateral microinfusions of lidocaine into the medial prefrontal cortex of rats immediately before testing on a recency discrimination task resulted in impaired performance (Hannesson, Howlands & Phillips, 2004). This is suggestive of a contribution of the mPFC to retrieval of object temporal order memory. Given the consistent PFC related deficits seen in DS individuals on tasks for set-shifting, working memory and inhibition, then it would follow that DS individuals may have problems with memory for temporal order. However, the lack of deficit seen in the Tc1 mice on the temporal order task is in broad agreement with a study which tested the ability of DS individuals to recall correctly ordered information using two auditory tasks, and a nonverbal, visual task. Although subjects with DS recalled significantly less information than mentally age matched controls, there was no significant difference in the ordering of recalled information (Bird & Chapman, 1994). Thus, it appears that within the context

of recognition memory within the Tc1 mouse model, there is intact function of the mPFC. However, DS individuals demonstrate more consistent deficits on tasks of executive function. For example, (Lanfranchi et al., 2010) carried out a battery of tests of executive function in 15 DS adolescents and 15 mentally age matched controls. The results revealed that the group of individuals with DS performed at a significantly lower level on each of the tasks for set-shifting, working memory and inhibition, consistent with altered pre-frontal functions in DS. Thus, it would be interesting to test Tc1 mice on tasks that map more specifically onto the executive function roles of the mPFC such as the rodent version of the stroop task, the stop-signal reaction task, or the Go/No-Go discrimination task, in order to test the integrity of this brain structure.

The pattern of findings relating to object recognition memory in Tc1 and Tg2576 mice are consistent with the hypothesis that dissociable synaptic processes underpin short-term and long-term recognition memory (Barker et al., 2006). Spatial recognition memory of Tc1 mice is unimpaired, with ability on object-in-place tasks both immediately and after 24-hrs, and performance on the object location task after 10-mins remaining intact. The ability to perform spatial, but not object recognition tasks points towards a problem with the perirhinal cortex, as opposed to the hippocampus. Additionally, the delay dependent pattern of memory, may suggest the involvement of the kainate, or muscarinic cholinergic receptor mechanisms in the memory deficits seen in the Tc1 mice. Further studies investigating receptor expression will be useful in helping to establish the underlying mechanisms involved in the memory deficits seen in the Tc1 mice. Further studies which interrogate the mPFC on tasks of executive function as opposed to temporal order tasks recognition memory tasks would also give a clearer picture about the function of the mPFC in Tc1 mice. Further studies focusing on cortical changes in Tc1 mice and other segmental trisomy mouse models will help elucidate the mechanisms by which trisomy of genes on human chromosome 21 disrupt memory processes.

4. Recognition memory retrieval processes in Tc1 mice

Chapter Overview

This chapter examined whether Tc1 mice displayed a deficit in short term novel object recognition when retrieving object information from long term memory. The experiments within this chapter demonstrated that Tc1 mice show an impairment on the novel object recognition task 10- minutes after presenting a retrieval cue, using either familiar objects or exposure to the training context 24-hours after the initial exposure, relative to WT animals. Finally, the chapter presents patterns of neuronal activation in the perirhinal cortex and hippocampus following a memory retrieval cue, using the immediate early gene, *c-fos*. The results suggest aberrant perirhinal cortex activity, and aberrant activity in the CA3 region of the hippocampus, which adds support to and extends the findings of chapter 3.

4.1 Introduction

Morice et al. (2008) reported a deficit in short term (10 minute) novel object recognition memory, but intact long term (24 hour) object recognition memory. This finding was extended by Hall et al. (2016), (see chapter 3), and showed that Tc1 mice displayed intact immediate object recognition memory, and intact processing of spatial attributes of recognition memory. A similar short-term object recognition memory deficit was reported by O'Doherty et al. (2005). However the method used was different to that used by Hall et al., (2016). In the O'Doherty et al., (2005) study, mice received two sample phases on day one, followed by a further two sample phases on day two, before a novelty test was conducted 10-minutes after the last sample trial on day two. Despite the 24-hr interval between sample phases, Tc1 mice were impaired on the novel object recognition task following a 10- minute delay. This finding suggests that retrieval of object information into short-term memory from long-term memory instigates a temporal-related deficit in recognition memory. Thus, it also appears that Tc1 mice may have difficulty processing object information following it's retrieval from long term memory.

The key point here is that following a 24 hour period, the representation of an event should be in long term memory. Testing Tc1 mice after a 24-hr delay interval with a familiar and a novel object results in a normal object novelty preference. In contrast, if Tc1 mice are presented with a copy of a familiar object following a 24-hr interval, and then subsequently tested for a novelty preference 10-minutes later, Tc1 mice are impaired. This pattern of results implies that the maintenance of information in short-term memory following retrieval from long-term memory is problematic.

A useful theory for thinking about the dynamics of short and long-term memory processes is Wagner's sometimes-opponent process (SOP) model of learning (Wagner, 1981). In Wagner's SOP model (see Figure 4.1), a stimulus, such as an object, consists of a set of stimulus elements. In the absence of the objects, the elements are in an inactive state (Wagner, 1981). Upon the initial presentation of a stimulus, the stimulus elements enter a primary active (A1) state; at this stage the elements generate a strong response to the objects. The stimulus elements then decay into a secondary active (A2) state. Once in the A2 state, the elements generate less responding to the objects (Wagner, 1981). Thus, animals will show less responding to objects over time as stimulus elements decay into the A2 state.

There is only one route by which stimulus elements may enter the A1 state; by presenting the stimulus itself (route 1). There are however, two routes by which stimulus elements are able to enter the A2 state. One route is through decay from the A1 state, and this is

referred to as self-generated priming (route 2), as the priming occurs as a direct result of the stimulus. The second route to the A2 state depends upon previously formed associations. Associatively activated elements can enter the A2 state directly, bypassing the A1 state (route 4). The data from the O'Doherty et al., (2005) paper, in which re-exposure to the objects following a 24-hr delay caused a deficit in novel recognition memory following a 10-minute delay, is suggestive of a problem with self-generated priming.

To test the idea that retrieval of object representations in short-term memory may be problematic in Tc1 mice, the first experiment in this chapter (Experiment 1a) first attempted to establish the deficit in object recognition memory following retrieval from long term memory. The hypothesis was that re-exposure to the sample objects would result in the deficit in Tc1 mice following a 24-hr interval. A control group received exposure to only the sample context.

The results of experiment 1a unexpectedly showed that re-exposure to the experimental context alone was enough to result in a deficit in memory retrieval in Tc1 mice. Experiment 1b was designed to further test the idea that exposure to the sample context 10-min prior to a novelty test would disrupt novelty recognition. A control group received exposure to the sample context 24-hrs prior to the novelty recognition test.

The first two experiments confirmed that exposure to the sample context prior to the novelty recognition test phase, conducted 24-hrs after the original sample trial, was disrupted in Tc1 mice. This observation provided an opportunity to examine the neural circuitry activated by the putative reminder treatment in WT and Tc1 mice. More specifically, because the behaviour of the Tc1 and WT mice were comparable prior to the test trial, we used the context exposure manipulation to examine the pattern of activation of the immediate-early gene *c-fos* in the hippocampus and related structures. It is widely accepted that the perirhinal cortex plays a key role in processing object identity (Bussey & Saksida, 2005) and object familiarity/novelty discriminations (Brown & Xiang, 1998). Given the selective disruption of short-term item recognition memory in Tc1 mice, it would follow that there may be aberrant function of the perirhinal cortex in these mice. Additionally, perirhinal *c-fos* expression has been shown to increase in rodent brains in response to novel stimuli (Zhu, Brown, McCabe & Aggleton, 1995; Wan et al., 1999; Wan et al., 2004), and electrophysiological studies have shown that repeated presentation of objects, i.e., familiar stimuli, is associated with a reduction in perirhinal cortex neural activity (Xiang & Brown, 1998; Brown & Aggleton, 2001). Thus, experiment 2 was designed to investigate patterns of neuronal activation in the perirhinal cortex and hippocampus following memory retrieval, using the immediate early gene, *c-fos*.

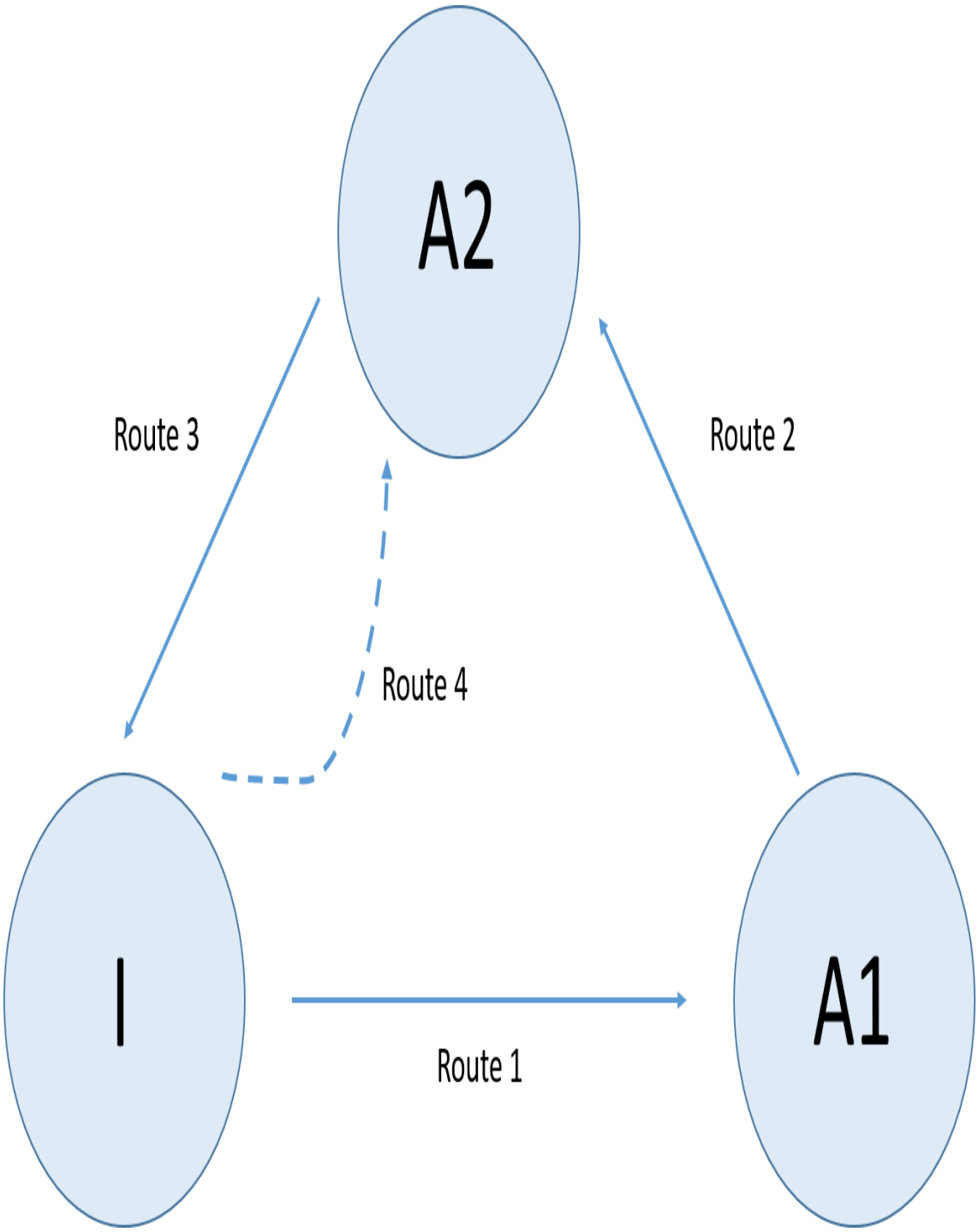


Figure 4.1- Schematic Diagram of Wagner's SOP *Diagram depicts the states in which a memory can reside; the primary active A1 state, the secondary active A2 state, and the inactive state. There are various routes by which memories can move between the states. Stimulus elements move from inactive to A1 upon stimulus presentation (route 1). Elements then decay into a secondary active A2 state whereby less responding is generated (route 2). The stimulus elements then decay into an inactive state (route 3). Stimulus elements cannot move directly from A2 back into A1. Associatively activated elements can enter the A2 state directly, bypassing the A1 state (route 4). Adapted from Sanderson et al (2010).*

4.2 Materials and Methods

4.2.1 Subjects

Mice were bred and transferred from The Francis Crick Institute as described in Chapter 3.

Experiments 1a and 1b were conducted using a cohort of 24 mice (12 WT and 12 Tc1). In experiment 1a, 1 WT and 1 Tc1 mouse were excluded from the task for failure to engage. Failure to engage with the task was defined as a zero contact time with the objects during the sample phases. Mice who failed to engage in the sample phases were not run through the test, and were excluded from all subsequent analysis. Experiment 2 was conducted using the same cohort of mice, however due to attrition, only 20 of the original animals were used (10 WT and 10 Tc1).

4.2.2 Apparatus

As described in Chapter 2.

4.2.3 Experimental Design

As described in Chapter 3, the order in which contextual manipulations were received were also counterbalanced.

4.2.4 Behavioural Methods

4.2.4.1 Experiment 1a: Tc1 Novel Object Recognition following 10-min after re-exposure to the experimental context

The main aim of this experiment was to establish whether the Tc1 mutation would disrupt novelty detection following re-exposure to the familiar cues 24 hours after initial exposure. The procedure was as described in chapter 2, however, following the second sample phase, mice were returned to their home cages for a 24- hour period. The next day, on day two, animals were given a third sample phase, whereby they could explore the arena and the objects again for 10-minutes (objects condition), or they were placed in the experimental arena, without the presence of objects (context condition) for 10-mins. The mice were then placed back in their home cages for a further 10-minutes, after

which the test phase took place. In the test phase, one of the items was replaced with a novel object (Figure 4.2). In addition to contact times and discrimination ratios, the locomotor activity of mice during the re-exposure phase was measured from the DVD recordings following the experiments. A 12.5x12.5cm square was drawn on an acetate which was placed over the outline of the arena on the screen. The square was divided into a 5x5 grid, with each grid square measuring 2.5x2.5cm. A tally was counted each time the head and front paws of the mouse crossed the boundaries between two squares of the grid in order to attain a crude measure of locomotor activity.

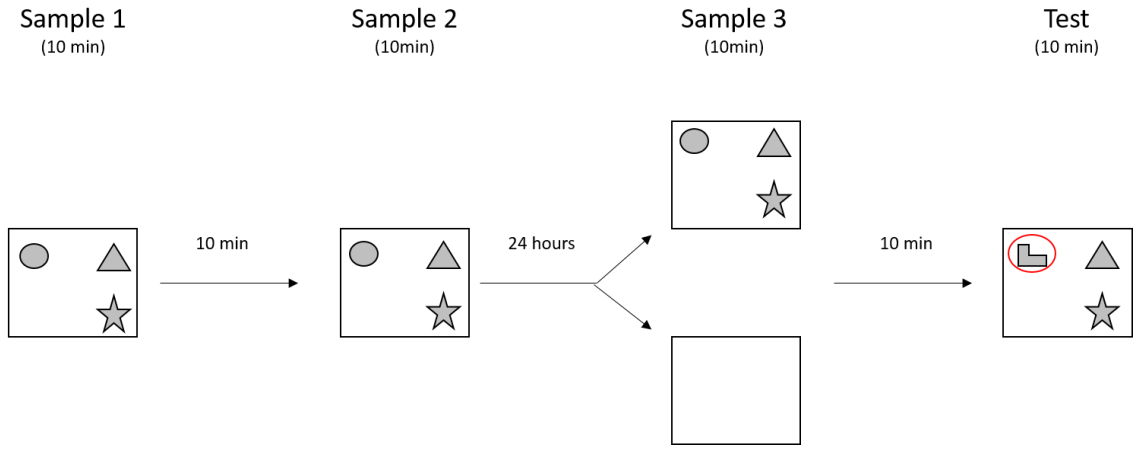


Figure 4.2 – Schematic representation of experiment 1a *– mice are exposed to three objects during two, 10- minute sample phases. Following 24-hrs back in their home cages, mice are re-exposed for 10-mins, to either the objects, or the empty box. After 10- minutes, mice are returned to the arena for the test phase, during which, one of the objects is replaced for a novel object.*

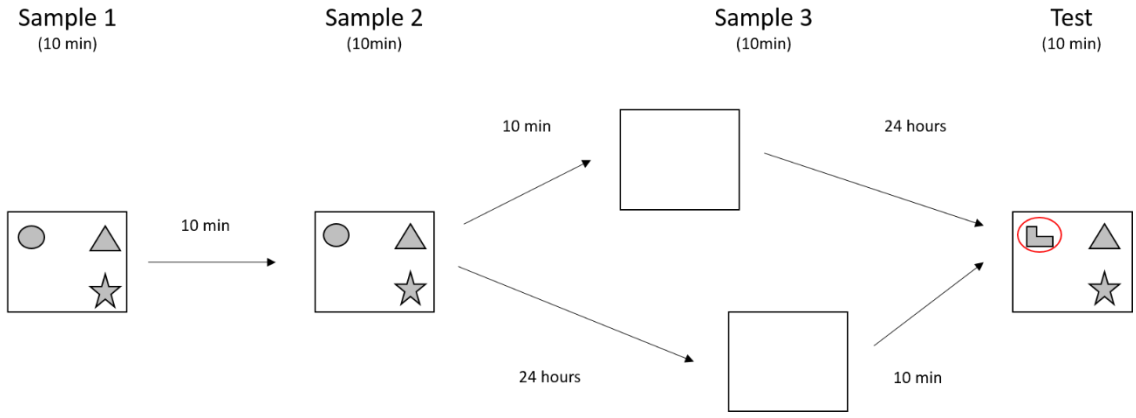


Figure 4.3 –Schematic representation of experiment 1b: *Task Design – mice are exposed to three objects during two, 10- minute sample phases. After a delay of either 10-mins or 24-hrs whereby mice are returned to their home cages, mice are re-exposed to the experimental context for 10- minutes. After a 10- minute or 24-hrs delay, mice are returned to the arena for the test phase, during which, one of the objects is replaced for a novel object.*

4.2.4.2 Experiment 1b: Tc1 Novel Object Recognition at 10-mins vs. 24 hours following re-exposure to the experimental context.

To establish whether the 24-hr novelty detection deficit in Tc1 mice following exposure to the sample context 10-mins prior to the test was related to a recent presentation of the context, we compared the effects of a short 10-min delay interval, with a long 24-hr interval between context re-exposure and the test phase. Following two sample phases, mice were placed back in their home cages for either 10-mins or 24- hours. Those placed in their home cages for 10- minutes were then placed back in the experimental context for 10-minutes, before being placed back in their home cages for 24- hours prior to the test phase. Those placed in their home cages for 24- hours, were placed back into the experimental context for 10- minutes, before being placed back in their home cages for 10- minutes prior to the test phase (Figure 4.3). In the test phase, one of the items was replaced with a novel object. The order in which mice received the 10-minute, or 24-hr delay prior to context re-exposure was fully counterbalanced. Discrimination ratios were calculated in the same manner as the previous experiment.

4.2.4.3 Experiment 2: c-fos Expression

C-fos is an immediate early gene (IEG) meaning that it is a gene which does not require previous protein synthesis to be activated, and it is assumed to have a role in long term plasticity by regulating downstream genes (Herrera & Robertson, 1996). Transient expression of *c-fos* in the brain was first seen following seizure activity, and *c-fos* expression is now widely used as an indirect marker of neuronal activity due to its wide distribution throughout the brain, and its low baseline levels which rapidly rise following behavioural interventions (Kinnavane, Amin, Horne, & Aggleton, 2014).

4.2.4.3.1 Behaviour:

In order to assess the role of the perirhinal cortex, and hippocampus on the behavioural phenotype shown in Tc1 mice, the time dependent context re-exposure task was carried out. Instead of the test phase, animals were placed in a dark room, and culled following 90 minutes; a 90 minute time period was used, as this is the time course for Fos protein expression (Chauduri, Zangenehpou & Rahbar-Dehghan, 2000). This allows for capture of the *c-fos* expression levels which represent those of the animal's brains at the test phase, in order to quantify regional brain activity when performing the task. Tc1 and WT mice were divided evenly into two behavioural groups – behavioural condition 1 and behavioural condition 2. Following two sample phases, mice were placed back in their home cages for either 10-mins or 24-hrs. Mice in condition one were placed in their home cages for 10-mins and were then placed back in the experimental context for 10-mins, before being placed back in their home cages for 24-hrs. Mice in condition two were placed in their home cages for 24-hrs, and were then placed back into the experimental context for 10-mins, before being placed back in their home cages for 10-mins. There will be no *c-fos* expression in response to manipulations 24 hours earlier, and so condition 2 was used as a control condition. Following the 24-hrs or 10-mins back in the home cages, mice were placed in a quiet, completely dark room, with a towel over their cages for 90 minutes prior to perfusion (Figure 4.4). The mice were habituated to this dark room for a 30 minute period on three consecutive days prior to testing.

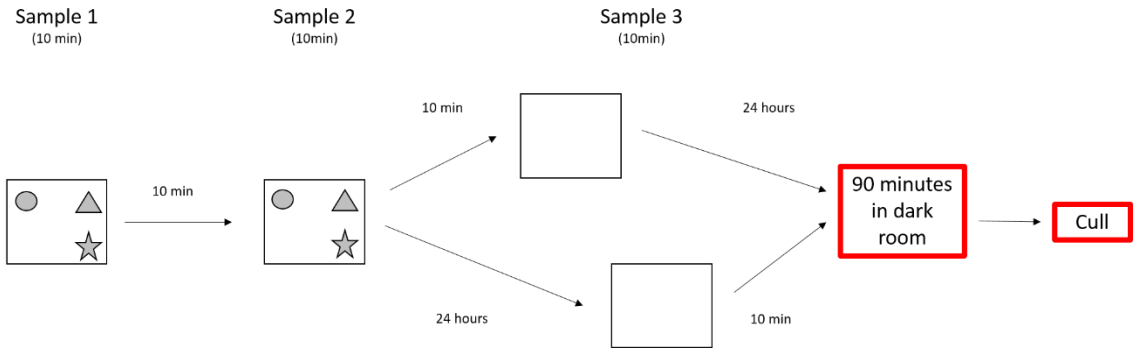


Figure 4.4 – Schematic representation of experiment 2: *Task Design – mice are exposed to three objects during two, 10- minute sample phases. After a delay of either 10-mins or 24 hours whereby mice are returned to their home cages, mice are re-exposed to the experimental context for 10- minutes. After a 10- minute or 24 hour delay, mice are placed in a dark room for 90 minutes directly before being culled, and the brain tissue processed for immunohistochemistry.*

4.2.4.3.2 Tissue preparation:

Mice were deeply anaesthetised via intra-peritoneal injection of 0.2ml of Euthatal (sodium pentobarbital). The mice were then exsanguinated, via insertion of a cannula into the left cardiac ventricle, with 80ml of phosphate buffered saline (PBS) (137mM NaCl, 1.5mM KH2PO4, 8mM Na2HPO4, 2.5mM KCl, pH 7.4). This was followed immediately by perfusion with 100ml of cold 4% paraformaldehyde in 0.01M PBS (PFA). The brain tissue was extracted from the skull and post-fixed for a further 8 hours in 4% PFA at room temperature on a stirrer plate, before being transferred to a solution of 25% sucrose in double distilled water (ddH_2O) for 48 hours at 4°C, until the brain tissue sank to the bottom of the solution. Tissue was mounted on a freezing microtome at -20°C and cut into 40µm coronal sections. A series of one in four sections was collected in PBS. Tissue sections were stored at -20°C in an ethylene-glycol based cryoprotectant until used for immunohistochemical analysis.

4.2.4.3.3 C-fos immunohistochemistry:

In order to reduce variability, a series from one mouse from each group were processed concurrently. First, sections were washed six times for 10- minutes in 0.2% Triton-X 100 in 0.1M PBS (PBST) in order to remove cryoprotectant. Sections were then washed once for 10- minutes in 0.3% H_2O_2 in PBST in order to block endogenous peroxidases, and then a further four times for 10- minutes in PBST. Sections were then incubated in primary antibody solution, polyclonal rabbit-anti-Fos diluted in PBST (1:15000; synaptic systems, cat no. 226 003), for 48 hours at 4° C. Sections were washed four times for 10-minutes in PBST, before being incubated in secondary antibody solution, biotinylated goat-anti-rabbit (1:200; Vector Laboratories) diluted in 1.5% normal goat serum in PBST for 2 hours at room temperature. Next, sections were washed four times in PBST, and incubated in avidin-biotinylated horseradish peroxidase complex in PBST (Elite kit, Vector Laboratories) for 1 hour at room temperature. Sections were then washed four times for 10- minutes in PBST, and then twice for 10- minutes in 0.05M Tris buffer. Finally, diaminobenzidine (DAB Substrate kit, Vector Laboratories) was used to visualize the reaction. This reaction was then stopped in cold PBS. Sections were mounted onto gelatin-coated slides, dehydrated in an ascending series of alcohol concentrations and cleared in xylene before being cover-slipped using Di-n-butyl phthalate (DPX) mounting medium.

4.2.4.3.4 Regions of interest:

The ROIs for *c-fos* analysis were the middle and caudal levels of areas 35 and 36 in the perirhinal cortex (AP -1.46 to -3.64). Borders were taken from Paxinos and Franklin (2004). Fos positive cell counts were also performed in the dorsal hippocampus (AP -1.46 to -2.18). The dorsal hippocampus was chosen as it performs a primarily cognitive role, in comparison to the ventral hippocampus which relates more to emotion and affect (Fanselow & Dong, 2010). The dorsal hippocampus has been implicated in spatial navigation and memory (Moser, Moser, Forrest, Andersen, & Morris, 1995). In contrast, lesions of the ventral hippocampus alone have no impact on the ability of mice to perform in a water maze, and additionally, lesions to the ventral hippocampus do not exacerbate the deficit caused by lesions to the dorsal hippocampus (Moser et al., 1995).

4.2.4.3.5 Image capture and analysis of c-fos activation:

For each ROI in each animal, images were captured from three sections, from both hemispheres with a 5x objective lens (numerical aperture of 0.12) on a Leica DMRB microscope and an Olympus DP70 camera. Counts were made in a field of view which was 0.84 x 0.63mm, and thus, all cortical regions of interest required only one image per section, hippocampal sections required multiple images which were stitched together using cell sense software (Olympus Life Science). To avoid experimenter bias, Fos positive cells were quantified using cellSens Dimensions software (Olympus Life Science), which selects and counts cells automatically. The Fos positive cell counts were determined by counting the nuclei (with a mean ferret, a measure of particle size, of 4–20 µm) which were stained above a greyscale threshold. The threshold was set ~ 70 units below the peak grey value for each image, and was measured from a pixel intensity histogram.

4.2.5 Data Analyses

Behaviour

Statistical analyses were conducted using SPSS (version 20.0). A two-way design was used with between subject factor of group and within subject factor of object type for contact time data. A repeated measures design using discrimination ratios as the within subject factor and genotype as the between subject factor were used for discrimination ratio data. Interactions were analysed using tests of simple main effects. The α-level was set at $p < 0.05$ for all comparisons. To compare discrimination values against chance, one-sample t-tests were carried out against a discrimination ratio value of 0.5.

IEG analyses

To analyse group differences in Fos-positive cells in the regions of interest, a two between-subjects factor (condition (10 mins or 24 hour hour delay after second exposure) and genotype) and one within-subject factor (ROI) ANOVA was performed. This analysis was carried out separately for two regional groupings: (i) Perirhinal cortex; and (ii) hippocampal subfields; CA1, CA3 and DG.

4.3 Results

4.3.1 Experiment 1a: Tc1 Novel Object Recognition at 10-mins following re-exposure to the experimental context, with the objects vs. re-exposure to the experimental context alone

The contact times for each group of mice during the first two sample phases (collapsed across both experimental conditions) are shown in Table 4.1 and contact times during the test phase in Table 4.2, respectively. Inspection of Table 4.1 indicates that Tc1 mice showed numerically higher contact times with the objects than WT mice. However, the duration of contact decreased in both Tc1 and WT at a similar rate across the sample phases. An Analysis of variance (ANOVA) with sample phase as the within subjects factor, and genotype as the between subjects factor revealed a significant main effect of sample phase on object contact time ($F_{(1, 20)} = 28.469$, $p<0.001$), but no significant main effect of genotype ($F_{(1, 20)} = 2.873$, $p = 0.106$), and no significant interaction between these variables ($F_{(1, 20)} = 1.609$, $p = 0.219$). This shows that both groups showed a significant decrease in activity (habituation) from sample phase 1 to sample phase 2.

Table 4.2 shows the mean contact times with objects (novel and familiar) across the delay conditions for Tc1 and WT mice. A repeated measures ANOVA using object and condition (objects or arena) as the within subjects factors, and genotype as the between subjects factor revealed a significant main effect of object ($F_{(1, 20)} = 40.827$, $p<0.001$) but no significant main effect of condition ($F_{(1, 20)} = 0.061$, $p = 0.807$). There is however a significant main effect of genotype ($F_{(1, 20)} = 6.046$, $p = 0.023$). The interaction between object and genotype, failed to reach statistical significance ($F_{(1, 20)} = 1.731$, $p = 0.203$). There was no significant interaction between object and condition ($F_{(1, 20)} = 0.987$, $p = 0.332$), no significant interaction between condition and genotype ($F_{(1, 20)} = 1.403$, $p = 0.250$), or three-way interaction between object, condition and genotype ($F_{(1, 20)} = 1.292$, $p = 0.269$).

In order to evaluate performance that was independent of individual differences in contact times, the data were also analysed using a discrimination ratio and are shown in Figure 4.5. Inspection of this figure indicates that wild type control mice discriminated between novel and familiar objects following re-exposure to the objects and arena, and following re-exposure to the arena alone. Tc1 mice were still able to discriminate between novel and familiar objects following re-exposure to the objects, and following re-exposure to the arena alone, to a level which was significantly above chance level. However, the performance was still reduced in comparison to that of WT mice. A repeated measures ANOVA using discrimination ratios as the within subjects factor and

genotype as the between subjects factor revealed a significant main effect of genotype ($F_{(1, 20)} = 7.078$, p=0.015), but no significant main effect of condition ($F_{(1, 20)} = 0.006$, p = 0.941). There was also no significant interaction between these factors ($F_{(1, 20)} = 1.446$, p = 0.243).

Furthermore, a one sample t-test confirmed that the performance of the WT mice were significantly above chance in both conditions (objects: $t_{(10)} = 3.794$, p =0.004; arena: $t_{(10)} = 10.364$, p <0.001). Although Tc1 mice performed at a level which was significantly worse than that of WT mice, they still performed significantly above chance level in both conditions (objects: $t_{(10)} = 3.616$, p =0.005; arena: $t_{(10)} = 3.218$, p =0.009).

These result therefore confirm that Tc1 mice showed a reduced memory for objects following retrieval from long term memory, following re-exposure to the objects, or the arena alone, compared to WT mice.

The locomotor activity was also measured during the re-exposure phase. The mean number of movements (a movement was counted each time the head and front paws of the mouse crossed the boundaries between two squares of the grid) of both Tc1 and WT mice during re-exposure to both the context containing the objects, and the context alone, can be found in Table 4.3. A repeated measures ANOVA using re-exposure condition (objects or context) as the within subjects factor and genotype as the between subjects factor revealed no significant main effect of genotype ($F_{(1, 20)} =0.066$, p=0.800), no significant main effect of re-exposure condition ($F_{(1, 20)} =0.105$, p=0.749), and no significant interaction between these factors ($F_{(1, 20)} = 0.231$, p= 0.636). This suggests the locomotor performance of the Tc1 and WT mice were comparable prior to the object novelty test.

Mean Contact Time During the Sample Phases (seconds)		
Genotype	**Experiment 1: Objects vs. Arena**	
	Sample Phase 1	Sample Phase 2
Tc1	37.47	26.44
WT	26.00	19.01
	Experiment 2: Re-exposure to arena 10 min vs. 24 hours prior to test	
	Sample Phase 1	Sample Phase 2
Tc1	36.68	25.80
WT	37.19	27.30

Table 4.1- Mean contact time during the sample phases (seconds) with all objects for Tc1 and WT control mice.

Mean Contact Times During the Test Stage (seconds)				
Genotype	**Experiment 1: Objects vs. Arena**			
	Novel Object (Object)	Novel Object (Arena)	Familiar Object (Object)	Familiar Object (Arena)
Tc1	12.88	9.83	6.39	5.95
WT	6.39	10.18	2.61	3.29
	Experiment 1b: Novel Object Recognition - 10minutes vs immediate delay			
	Novel Object (10mins)	Novel Object (24 Hour)	Familiar Object (10mins)	Familiar Object (24 Hour)
Tc1	14.24	17.75	16.93	10.05
WT	25.57	10.49	13.09	4.61

Table 4. 2 – Mean contact time during the test phase (seconds) with novel and familiar objects for Tc1 and WT control mice.

Mean number of movements during the re-exposure phase			
Objects		Context	
Tc1	WT	Tc1	WT
172.91	187.27	176.27	169.91

Table 4.3 Experiment 1a *– Mean number of movements during the re-exposure phase, following re-exposure to both objects and the context alone as a measure of locomotor activity in both Tc1 and WT control mice.*

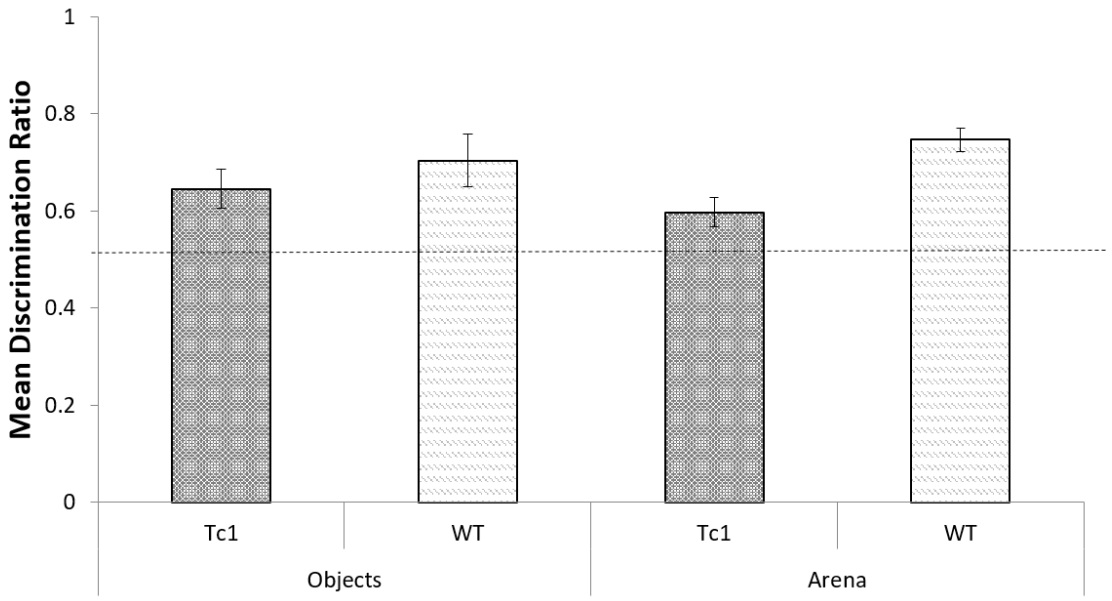


Figure 4.5– Results of experiment 1a *Novel object recognition following a re-exposure 10- minutes before the test phase, to either the context containing objects, or the context alone, 24 hours after the initial sample phases. Mean discrimination ratios (error bars represent ± SEM) describing the preference for the novel object for Tc1 and wild type (WT) mice. (---------- = 0.5, chance).*

4.3.2 Experiment 1b: Tc1 Novel Object Recognition at 10-mins vs. 24 hours following re-exposure to the experimental context.

The main aim of experiment 1b was to investigate whether the memory deficit which occurred when using the experimental context (arena), was sensitive to the same delay dependent differences as the simple novel object recognition deficit. The mean contact times shown by WT and Tc1 mice during the sample phases are shown in Table 4.1. An ANOVA, with sample phase as the within subject factor and genotype as the between subject factor, revealed a significant main effect of sample phase on contact times ($F_{(1, 22)} = 39.771$, $p < 0.001$), but no significant main effect of genotype ($F < 1$, $p = 0.770$), and no significant interaction between these variables ($F < 1$, $p = 0.765$). This analysis confirmed that both groups interacted with the objects at the same rate and habituated to the stimuli across the sample phases.

The mean object contact times during the novelty recognition test are shown in Table 4.2. Inspection of this table shows that Tc1 mice displayed a normal object novelty preference when tested twenty-four hours after the arena re-exposure, but not after a 10-minute interval between arena re-exposure and the novelty test. A repeated measures ANOVA, with object type (novel and familiar), and delay (ten minutes and twenty four hours) as within subjects factors and genotype as a between subjects factor, revealed no significant main effect of genotype ($F_{(1, 22)} = 2.562$, $p = 0.124$). There was, however, a significant main effect of object, ($F_{(1, 22)} = 32.627$, $p<0.001$) and a significant interaction between these factors ($F_{(1, 22)} =15.329$, $P=0.001$). Tests of simple main effects revealed a significant effect of genotype on contact times with the novel object ($F_{(1, 22)} =7.272$, $p=0.013$), but not with the familiar object ($F_{(1, 23)} = 0.852$, $p=0.366$). There was a significant simple effect of object type for the wild type mice ($F_{(1, 23)} = 46.342$, $p<0.001$), but not for the Tc1 mice ($F_{(1, 23)} = 1.614$, $p = 0.217$).

In addition, the ANOVA revealed there was no significant main effect of the delay ($F_{(1, 22)} = 0.635$, $p= 0.434$), and no significant delay x genotype interaction ($F_{(1, 22)} <0.001$, $p= 0.985$). There was, however, a significant object x delay interaction ($F_{(1, 22)} = 14.764$, $p=0.001$). Tests of simple main effects revealed an effect of object at both delays, at ten minutes ($F_{(1, 22)} = 43.072$, $p<0.001$), and 24 hours ($F_{(1, 22)} = 10.253$, $p = 0.004$). There was no significant simple effect of delay on contact times with either the novel ($F_{(1, 22)} = 0.532$, $p = 0.473$) or the familiar object ($F_{(1, 22)} = 3.726$, $p = 0.067$).

Finally, there was a significant context delay x object x genotype interaction ($F_{(1, 22)} = 7.033$, $p=0.015$). There was no significant simple effect of context delay on the Tc1 animals interaction with the novel object ($F_{(1, 22)} = 1.607$, $p =0.218$), or with the familiar object ($F_{(1, 22)} = 3.960$, $p = 0.059$). There was no significant simple effect of context delay

on the wild type animals interaction with the novel object ($F_{(1, 22)} = 0.056$, $p= 0.815$), or the familiar object ($F_{(1, 22)} = 0.547$, $p = 0.467$).

Simple effects tests revealed a significant simple effect of genotype on the interaction with the novel object in the 24 hour condition ($F_{(1, 22)} = 4.699$, $p=0.041$), and a significant simple effect of genotype on the interaction with the novel object in the 10-min condition ($F_{(1, 22)} = 6.341$, $p= 0.020$). There is no significant simple effect of genotype on the interaction with the familiar object in the 24-hr condition ($F_{(1, 22)} = 0.086$, $p=0.772$), or in the 10-min condition ($F_{(1, 22)} = 0.928$, $p=0.346$).

There was only a significant simple effect of object for the Tc1 animals in the 24 hour condition ($F_{(1, 22)} = 10.476$, $p= 0.004$), but not in the 10 minute condition ($F_{(1, 22)} = 1.537$, $p=0.228$), whereas there is a significant simple effect of object for the wild type animals in both the 24 hour condition ($F_{(1, 22)} = 36.556$, $p<0.001$), and the 10 minute condition ($F_{(1, 22)} = 33.273$, $p<0.001$).

The exploration time of the animals was then converted into discrimination ratios, calculated as the time spent exploring novel objects/ total time spent exploring objects and can be seen in Figure 4.6. A repeated measures ANOVA using discrimination ratios as the within subjects factor and genotype as the between subject factor revealed a significant main effect of genotype ($F_{(1, 22)} =25.814$, $p<0.001$), but no significant main effect of condition ($F_{(1, 22)} =16.280$, $p=0.001$). There was however, a significant interaction between these factors ($F_{(1, 22)} = 7.376$, $p= 0.013$). Simple main effects tests revealed no significant simple effect of genotype when the context exposure occurred 24- hours before the test ($F_{(1, 22)} = 1.819$, $p = 0.191$), but there was a simple effect of genotype when the context exposure was given 10- minutes before the test ($F_{(1, 22)} = 34.448$, $p <0.001$). There was a significant simple effect of condition on the Tc1 animals ($F_{(1, 22)} = 22.786$, $p<0.001$), but not on the wild type animals ($F_{(1, 22)} = 0.870$, $p=0.361$).

Furthermore, one sample t-test confirmed that the performance of the WT mice were significantly above chance level on both delay conditions (10-min: $t_{(11)} = 5.214$, $p <0.001$, 24 hour: $t_{(11)} = 5.666$, $p <0.001$). Tc1 mice in the 10-min condition performed significantly below chance level ($t_{(11)} =2.712$, $p =0.02$) whereas Tc1 mice in the 24-hr context exposure condition performed significantly above chance level ($t_{(11)} = 6.537$, $p < 0.001$).

These data demonstrate that Tc1 mice show a deficit following retrieval of memories by way of a context prime, following a short 10- minute delay, but not when the context prime is delivered 24-hrs prior to the test. Thus, the context priming deficit is sensitive to the same delay dependent conditions as the initial object recognition deficit observed by Morice et al., (2008), and Hall et al., (2016).

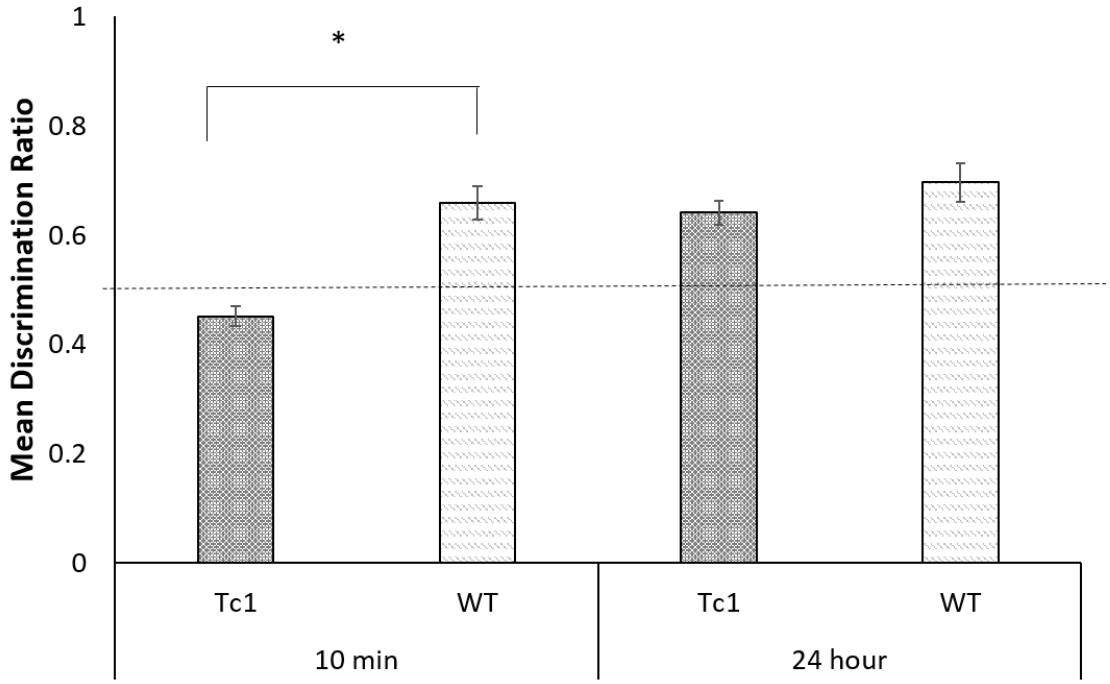


Figure 4.6 – Results of experiment 1b- *Novel object recognition following re-exposure to the context alone either 24-hr or 10-min after the initial sample phases in Tc1 and WT control mice, followed by a test 10-min or 24-hr after the re-exposure. Mean discrimination ratios (error bars represent ± SEM) describing the preference for the novel object for Tc1 and wild type (WT) mice (* = $p < 0.05$, ---------- = 0.5, chance).*

4.3.3 Experiment 2: Context Re-exposure –c-fos

Transient expression of *c-fos* in the brain is widely used as an indirect marker of neuronal activity (Kinnavane, Amin, Horner & Aggleton, 2014). The aim of this experiment was to indirectly investigate the pattern of neural activity in the hippocampus and perirhinal cortex of Tc1 and WT mice in response to context re-exposure, a procedure that should elicit a representation of the familiar objects presented in the environment (Aggleton and Brown, 2005; Wilson et al., 2013).

Hippocampus

An example of *c-fos* staining in the hippocampus of Tc1 and WT mice at the 10-min delay can be seen in Figure 4.8. For the hippocampus: Number of Fos positive nuclei found in each sub-region of the hippocampus (CA1, CA3 and DG) in Tc1 and WT mice can be seen in Figure 4.7. A repeated measures ANOVA with ROI; CA1, CA3 and DG, as the within subjects factor, and delay condition; 10- minutes and 24- hours, and genotype as between subjects factor revealed a significant main effect of ROI ($F_{(1, 16)} = 36.078$, $p < 0.001$), but no significant main effect of genotype ($F_{(1, 16)} = 0.746$, $p = 0.401$), and no significant main effect of delay condition ($F_{(1, 16)} = 0.583$, $p = 0.456$). The interaction between ROI and genotype failed to reach significance ($F_{(1, 16)} = 1.907$, $p = 0.183$), as did the interaction between ROI and delay condition ($F_{(1, 16)} = 0.436$, $p = 0.654$). There was however a significant interaction between genotype and delay condition ($F_{(1, 16)} = 7.348$, $p= 0.015$). Tests of simple effects revealed a significant effect of genotype at the 24- hour delay ($F_{(1, 16)} = 6.388$, $p =0.022$), but not at the 10- minute delay ($F_{(1, 16)} = 1.706$, $p = 0.210$).

There was also a significant three way interaction between ROI, genotype and delay condition ($F_{(1, 16)} = 4.009$, $p = 0.040$). Tests of simple main effects revealed a significant effect of genotype at the 24- hour condition in the CA1 region of the hippocampus ($F_{(1, 16)} = 8.016$, $p =0.012$), but not in the CA3 ($F_{(1, 16)} = 3.394$, $p = 0.084$), or the DG ($F_{(1, 16)} = 1.973$, $p = 0.179$). There was also a significant effect of genotype at the 10-min condition in the CA3 region of the hippocampus ($F_{(1, 16)} = 5.378$, $p = 0.034$), but not in the CA1 ($F_{(1, 16)} = 0.715$, 0.410) or the DG ($F_{(1, 16)} = 0.847$, $p = 0.371$). Tests of simple main effects also revealed a significant effect of delay condition on the expression levels of *c-fos* in the CA1 of Tc1 animals ($F_{(1, 16)} = 5.763$, $p = 0.029$), but not in the CA1 of WT animals ($F_{(1, 16)} = 1.629$, $p = 0.220$). There was also a significant effect of delay condition on the expression levels of *c-fos* in the CA3 of Tc1 animals ($F_{(1, 16)} = 5.318$, $p =0.035$), but not the CA3 of WT animals ($F_{(1, 16)} = 3.442$, $p = 0.082$). There was no significant effect of delay condition in the DG of Tc1 animals ($F_{(1, 16)} = 3.045$, $p = 0.100$) or WT animals ($F_{(1, 16)} = 0.336$, p 0.570).

Perirhinal Cortex

An example of *c-fos* staining in the perirhinal cortex of Tc1 and WT mice following the ten-minute and 24-hr delay conditions is shown in Figure 5. The mean number of Fos positive nuclei in the perirhinal cortex of Tc1 and WT mice is summarised in Figure 6. A univariate ANOVA with re-exposure delay condition; ten minutes and twenty-four hours, and genotype as independent variables, and *c-fos* counts in the perirhinal cortex as the dependent variable revealed no significant main effect of genotype ($F_{(1, 16)} = 0.735$, $p = 0.404$), and no significant main effect of delay condition ($F_{(1, 16)} = 0.010$, $p = 0.922$). There was however a significant interaction between genotype and delay condition ($F_{(1, 16)} = 5.334$, $p = 0.035$). Tests of simple effects revealed a significant effect of genotype on the expression of Fos positive nuclei in the perirhinal cortex at the ten-minute delay ($F_{(1, 16)} = 5.014$, $p = 0.004$) but not at the twenty-four hours delay condition ($F_{(1, 16)} = 1.055$, $p = 0.320$).

In Summary

These analyses suggest significant increases in perirhinal cortex and hippocampal CA3 region *Fos* expression in Tc1 mice 10-mins after the animals were re-exposed to the sample context following a 24-hr delay. The data also suggest a significant increase in CA1 activity 24-hr after the context re-exposure. However, due to the lack of measurement of *Fos* expression in a control region which is not immediately involved in recognition memory in order to determine baseline levels of *c-fos*, no solid conclusions can be drawn.

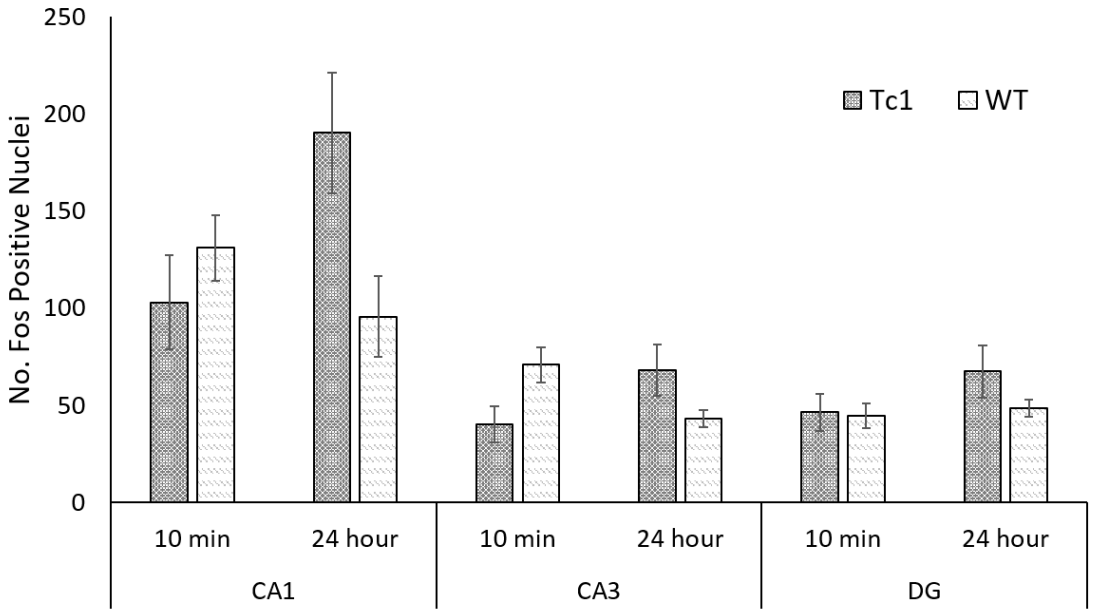


Figure 4.7- Number of Fos positive cells in the hippocampus *- CA1, CA3 and Dentate Gyrus – of Tc1 and control mice.*

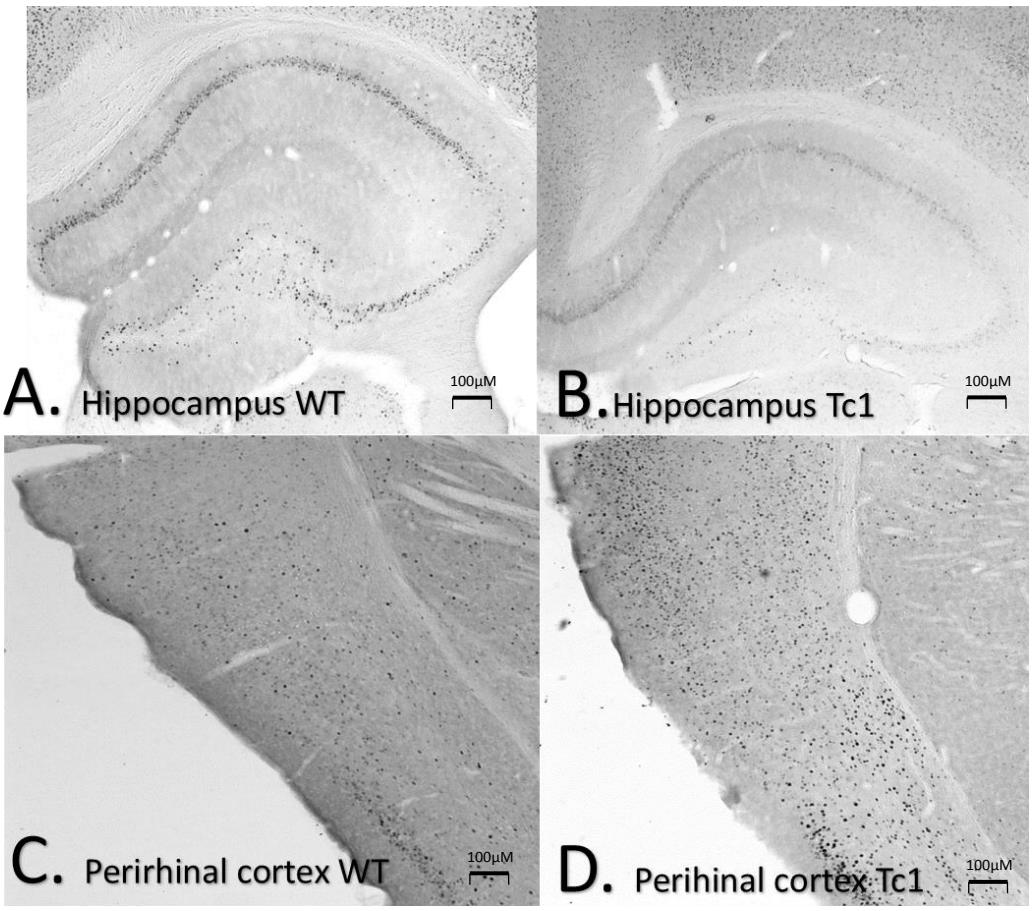


Figure 4.8– Examples of Fos positive staining *in the hippocampus and perirhinal cortex of Tc1 and WT control mice.*

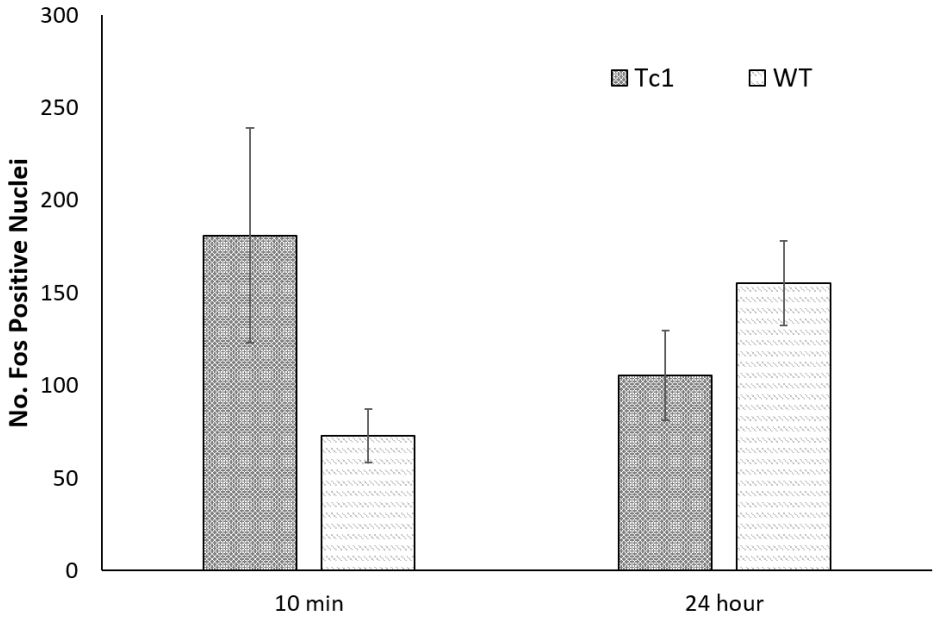


Figure 4.9 – Number of Fos positive cells in the perirhinal cortex *of Tc1 and WT control mice.*

4.4 Discussion

Tc1 mice are impaired in short term (10-min) but not immediate or long-term (24-hr) recognition memory (Morice et al., 2008; Hall et al., 2016). The present results extend these findings, demonstrating that not only do Tc1 mice display a deficit in short term novel object recognition, but that they also demonstrate an impairment in discriminating between novel and familiar objects following a 24-hr delay when either presented again with the sample objects or exposed to the context in which the objects were presented, relative to WT animals. In experiment 1a, both Tc1 and WT animals performed above chance level, in contrast to Tc1 performance in chapter 3, where Tc1 mice performed consistently significantly below chance level. This may reflect the heterogeneity of the transchromosome expression, or may reflect the fact that the retrieval of information is not as susceptible to the short term memory deficit. However, upon inspection of the data from experiment 1b, it is clear that Tc1 performance was not significantly above chance level at the ten minute delay condition, which seems to suggest that re-exposure is susceptible to the short term memory deficits alluded to in earlier chapters. However, the variability of gene expression within the Tc1 mouse line cannot be ruled out.

More specifically, these findings suggest that when object information is retrieved from long-term memory into short-term memory following a 24-hr delay, either by representing the objects or by using the context as a reminder cue, Tc1 mice subsequently show impaired discrimination between novel and familiar objects when the recognition test is conducted 10-mins but not 24-hrs later, relative to WT animals. The context re-exposure procedure was then used to examine how this manipulation influenced neural activity indexed by the expression of the immediate early gene *c-fos.* This experiment showed that recent exposure to a sample context resulted in significantly higher expression of *c-fos* in the perirhinal cortex of Tc1 mice than that in WT mice. In contrast *c*-fos expression 24-hours after context exposure was comparable between Tc1 and WT mice. In the hippocampus, recent exposure to the sample context resulted in lower *c-fos* expression in the CA3 region in Tc1 mice relative to WT mice. The implication of these results for perirhinal and hippocampal function in Tc1 mice will be considered after discussion of the main behavioural findings.

The findings by O'Doherty et al., (2005) are in agreement with the findings of Morice et al., (2008) in that both papers describe a short-term (but not long-term) deficit in object recognition memory in Tc1 mice. However, there were subtle but important differences in methodology between the two studies which have theoretical importance. Morice et al., (2008) showed impaired recognition in Tc1 mice following a 10-minute interval between sample and test. In contrast, Tc1 mice showed normal recognition following a

24-hr delay interval. O'Doherty et al., (2005) showed impaired recognition memory following a 10-minute delay when the sample trials were separated by 24-hrs. One interpretation of this latter finding is that the Tc1 mutation disrupts short-term memory processes following both encoding of information and also following retrieval of information from long-term into short-term memory. Experiment 1a and 1b replicated and extended the findings by showing that presentation of the sample objects or the context after a 24-hr delay disrupted recognition memory in Tc1 mice after a 10-min delay.

How might these results inform our theoretical understanding of short- and long-term memory processes in Tc1 mice? As described in the introduction, one prominent theory of learning describes an interaction between different memory states that can explain both short and long-term habituation and the influence of context on memory and performance. In Wagner's SOP model, a stimulus, such as an object, consists of a set of stimulus elements. In the absence of the objects, the elements are in an inactive state equivalent to long-term memory (Wagner, 1981). Upon the initial presentation of a stimulus, the stimulus elements enter a primary active (A1) state; at this stage the elements generate a strong response to the objects. The stimulus elements then decay into a secondary active (A2) state. Once in the A2 state, the elements generate less responding to the objects (Wagner, 1981). Thus, animals will show less responding to objects over time as stimulus elements decay from the A1 state into the A2 state. So, in the language of SOP, 24- hours after the first two sample phases, re-exposure of the mice to the objects or context would prime the elements of the familiar object back into A2; the elements in A1 would decay into A2, thus when the object is presented again, more of the stimulus elements are in A2, and generate weaker responding. Thus, in the test phase of a novel object recognition task, after a short delay, animals should show a preference towards the novel object. This would occur as a result of the stimulus elements of the novel object entering the primary active A1 state, whereby strong responding is generated. In comparison, the stimulus elements of the familiar object would be primarily in the A2 state as a result of its recent presentations (self-generated priming) and from context based associative priming.

This analysis suggests that in Tc1 mice there are a higher proportion of familiar stimulus elements available in the A1 state. This suggests a common cause of the short term recognition memory deficit in the Tc1 mouse; the inability of Tc1 mice to retain stimulus elements in the A2 state. This may result from a slower decay of stimulus elements from the A1 to the A2 state, or a more rapid decay of elements from the A2 state into the inactive state. In fact this theory also speaks to the results of chapter 3, whereby Tc1 mice display intact recognition memory performance at the immediate time point, in addition to the 24 hour time point. If the problem is in fact with the rate of degradation of

stimulus elements out of the A2 state, then it may be that the immediate time point is reflective of the stimulus elements remaining in the A2 state for a brief period. Whilst using Wagner's SOP however provides a good mechanistic explanation as to what is happening during the test phase, it provides a challenge as to why Tc1 mice show intact habituation across the sample phases. Further discussion of this issue will be reserved for the general discussion.

Another major caveat of using Wagner's SOP as a tool for explaining the data, is that it implies specificity of the context the objects are presented in. An alternative view to Wagner's SOP with regards to the results attained in the context re-exposure condition is that there is an interference effect that influences performance in Tc1 mice. Interference refers to situations in which a subject learns information at one stage, which can interfere with information learned at another stage (Bouton, 1993). When investigating classical Pavlovian conditioning, extinction is one of the most commonly studied retroactive interference paradigms, which involves destruction of the original association between a conditioned stimulus and an unconditioned stimulus. However, spontaneous recovery has often been noted, whereby if time is allowed to elapse following extinction, then the extinguished response recovers (reference). This suggests that the original learning still exists, and the passage of time may control this recovery to some extent, thus performance is only temporarily suppressed. The 'renewal effect', is where a conditioned stimulus is paired with an unconditioned stimulus in context A, and then presented alone in context B so as to extinguish responding in context B (reference). When the conditioned stimulus is then presented in context A again, there is renewed responding to the conditioned stimulus. Thus context also has an important impact on performance following interference. Bouton (1993) suggests that extinction is a retrieval problem, whereby representations of both phases are stored and are ready to be retrieved by the context, and what determines subsequent performance is the extent to which each representation is retrieved.

If we think about experiment 1b in terms of an interference paradigm, during the first two sample phases, both WT and Tc1 mice habituate, and therefore spend less time exploring the objects in sample phase two than in sample phase one. Upon re-exposure to the context alone however, the expectations of the mice are not met, in that there are no objects present in a context they have learnt to associate with objects, thus inducing a prediction error. It could be then that this memory of the context alone goes on to compete with the memory of the context with objects present. As Bouton (1993) suggested, representations of both the context containing objects, and of the context alone, would be stored, and the subsequent performance of the mice would then be determined by the extent to which each representation is retrieved. The data would

suggest that the Tc1 mice are more sensitive to this interference effect than WT animals, potentially being more likely to retrieve the representation of the context alone, and so finally in the test phase, when the context is presented with objects again, the Tc1 mice may be unable to distinguish between novel and familiar objects due to this interference, and demonstrate a deficit on the novel object recognition paradigm.

If it were the case, however, that the deficit in the Tc1 mice was as a result of an interference effect, we may expect to see some change in locomotor activity in the mice during the re-exposure phase, which would reflect their aberrant response to the prediction error they are experiencing. However, if we look at the locomotor data during the re-exposure to both objects and context, we can see that there is no significant difference between Tc1 and WT animals. There is also no difference in locomotor activity dependent on whether the animals are being re-exposed to the context containing objects, or just to the context alone. Thus, if there is prediction error during re-exposure which is great enough to cause interference, it is not affecting exploration prior to the test phase. In that Tc1 mice are not experiencing altered locomotor activity in the re-exposure phase, therefore they have the same opportunity to encode during this phase as WT animals do. Although locomotor and contact times increase with re-exposure, there are no differences between Tc1 and WT. Thus, based on this data, it is less likely that the deficit in the Tc1 mice is as a result of interference. This analysis is only useful when thinking about the context re-exposure, however, and is unable to explain the deficit which is seen following re-exposure to the objects, whereby there would be no prediction error.

Of course, in order to more confidently rule out an interference effect, and to use Wagner's SOP as a more definitive explanation, an additional control group would be required, whereby mice were exposed to an alternative context. It would be hypothesised that mice re-exposed to the experimental context 10- minutes prior to the test, would display a deficit on the novel object recognition task, whereas those exposed to an alternate context would show intact performance. This would be the anticipated result because the alternative context would not engage the associative priming route, or the self-generated priming route. If of course both groups of mice were affected, with both those exposed to the experimental context, and those exposed to the alternative context experiencing a deficit in the test phase, this would provide more support for the interference theory. Winters et al., (2006) has suggested that an event which takes place within the retention delay may interfere retroactively with the memory trace, and disrupt object recognition. For example, when infusing scopolamine, a cholinergic muscarinic receptor antagonist into the perirhinal cortex of rats within the retention delay, Winters, Saksida and Bussey et al., (2006) found that this not only failed to impair object

recognition memory, but actually facilitated performance relative to trials on which rats received saline infusions at the same time points. Winters, et al., (2006) suggested that the particularly poor performance of rats receiving saline infusions within the retention delay might be the manifestation of an interference effect, which was blocked by scopolamine infusions. In fact, they found that simply omitting the procedure of infusing saline recovered object recognition performance to normal levels. Thus, the act of doing something unexpected, such as being placed in an unexpected context, may also interfere with the original memory trace, thus causing a deficit in the test phase.

It is also important to consider the anatomical substrates of the retrieval of recognition memories in rodents and its implications for our understanding of memory systems in Tc1 mice. It is generally accepted that the perirhinal cortex plays a key role in processing object identity (Bussey & Saksida, 2005) and object familiarity/novelty discriminations (Brown & Xiang, 1998). Given the selective disruption of short-term item recognition memory in Tc1 mice it would follow that there may be aberrant function of cortical systems supporting object memory, namely the perirhinal cortex. Additionally, perirhinal *c-fos* expression has been shown to increase in rodent brains in response to novel stimuli (Zhu et al., 1995; Wan et al., 1999, Albasser, Poirer & Aggleton, 2009, Aggleton & Brown, 2005), and electrophysiological studies have shown that repeated presentation of objects, thus familiar stimuli, are associated with a drop in *c-fos* expression in the perirhinal cortex (Xiang & Brown, 1998; Brown & Aggleton, 2001). In experiment 2, Tc1 mice failed to show a reduction in perirhinal cortex *c-fos* expression following re-exposure to the experimental context 10-mins prior to the test phase. If in WT mice, low perirhinal *c-fos* activity is indicative of a familiarity response, then the lack of *c-fos* suppression in the perirhinal cortex of Tc1 mice is consistent with the hypothesis that the deficit in short term object novelty discrimination may lie with abnormal perirhinal cortex activity in the Tc1 mouse. This lack of perirhinal cortex suppression may explain why Tc1 mice treat familiar objects as novel following re-exposure to the experimental context 10-mins prior to test.

The present study also revealed changes in *c-fos* expression in sub-regions of the hippocampus. Computational models suggest that the anatomy and physiology of the hippocampus is appropriate for encoding and storage of a large number of associations (Marr, 1971; McNaughton & Morris, 1987; Rolls & Treves, 1998). In terms of sub-region function, Rolls (1987) suggested that CA3 neurons operate as an auto-association memory to store episodic memories, including object and place information, and that the dentate gyrus acts as a pre-processing stage for this by performing pattern separation to set up different representations for each memory to be stored in the CA3 cells. Rolls (1987) suggested that the CA1 cells operate as a re-coder for the information recalled

from the CA3 cells to a partial memory cue. The ability to recall a whole memory from only a partial cue is called pattern completion (Rolls, 2013), and contextual information is an important component of memory retrieval processes.

Rolls (2013) has suggested that the CA3 region of the hippocampus is responsible for implementing an auto-association memory between spatial context, and object representations, so that upon presentation of just part of the memory, pattern completion of the memory can occur within the CA3 network. A significantly lower level of *c-fos* expression was observed in the CA3 sub region of the hippocampus in Tc1 mice in the 10--minute delay condition following re-exposure to the context. This reduced activity within the CA3 network may underpin the inability of the Tc1 mice to perform pattern completion processes. It could perhaps be speculated upon that the A2 state from Wagner's model may be implemented by the CA3 region of the hippocampus, and that dysfunction of the CA3 region thus disrupt activation of A2 state; they are unable to undergo pattern completion. Another fundamental property of the pattern completion model of the CA3 is that it can implement short-term memory by maintaining the firing of neurons (Kesner & Rolls, 2015). Nevertheless, the lower level of *c-fos* expression in Tc1 mice at the 10-min delay indicates activation of this network is aberrant following re-exposure to the context and this may disrupt short-term or pattern completion processes supported by this network.

Rolls (1987) also suggested that the CA1 region can operate as a competitive network which is involved in the recall process. Back-projections to the cerebral cortex allow neuronal activity during recall to reflect the neuronal activity which was present during the original learning, making for efficient recall. Kesner and Rolls (2015) have more recently suggested that the role of the CA1, however, goes beyond simple recall, and that the CA1 makes a special contribution to temporal aspects of memory including associations over a delay period, sequence and temporal order memory, and, importantly to this experiment, the CA1 sub-region appears to have cellular processes that match it to longer term forms of memory compared to the CA3 region. For example, rats with CA1 lesions were impaired in retrieval with a 24 hour delay, but have no difficulty encoding new information, when tested in a modified Hebb-Williams maze (Kesner & Rolls, 2015). Tc1 mice showed significantly increased CA1 activity 24 hours after re-exposure to the experimental context relative to WT animals. This increase in *c-fos* expression may reflect a compensatory or delayed process and/or the use of alternate retrieval strategies. However, the role of the CA1 region in recognition memory and retrieval processes in this context has not been examined to date (Warburton & Brown, 2015) and thus any analysis of the conditions and mechanisms by which the CA1 region contributes to retrieval processes must remain speculative. One possibility is that that

short-term memory mediated by the CA3 and long term memory mediated by the CA1 can operate independently, and that Tc1 mice may have specific deficits related to aberrant CA3 function.

A major criticism of this work however, is the absence of measurement of *c-fos* expression in a control region which is not directly involved in recognition memory, in order to establish baseline levels of *c-fos* in the Tc1 mice. As a result, no solid conclusions can be drawn about the changes seen in *c-fos* in the perirhinal cortex and hippocampus. However, the changes seen in Tc1 mice are not uniform changes, and so it could be argued that this is suggestive that *c-fos* levels are not globally increased, or globally decreased within the brains of Tc1 mice.

In summary, Tc1 mice demonstrate an impairment on the NOR task, when compared to WT mice, following a short delay when retrieving object information from long term memory, either by re-exposure to the objects, or by re-exposure to the experimental context. No change in recognition memory was detected in Tc1 mice when a delay interval of 24-hrs intervened between the reminder treatment and novelty test. Finally, the *c-fos* analysis suggests potential aberrant CA3, CA1 and perirhinal cortex activity in the Tc1 mice, as quantified by *c-fos*. How these changes reflect altered retrieval or short-term memory processes remains unclear, and the addition of counts within a control region would make these changes clearer, but the data do suggest an interaction between the perirhinal cortex and hippocampus during contextual retrieval of object information. Further studies in both normal and Tc1 mice are required to understand the interactive nature of activity in the hippocampus and perirhinal cortex and how such dynamics are influenced by trisomy of chromosome 21 genes.

5. Glutamate receptors and short-term object memory in Tc1 mice

Chapter Overview

Previous research has demonstrated reduced surface expression of the GluR1 subunit of the AMPA receptor in the dentate gyrus of the hippocampus in Tc1 mice (Morice et al., 2008). In this chapter, experiment 1a first aimed to replicate this finding, and examine the expression levels of the GluR1 receptor in the perirhinal cortex. In chapter 4, evidence was presented that Tc1 mice showed a specific time-dependent deficit in novel object recognition, with deficits following a short ten minute delay, but not following a long 24 hour delay. Similar dissociations between short- and long-term recognition memory have been reported following manipulation of kainate receptors in the perirhinal cortex (Barker et al., 2006). Experiments 1b and 1c investigated the expression levels of GluK1 (GRIK1) and GluK5 (GRIK5) kainate receptor subunits in the hippocampus and perirhinal cortex of Tc1 mice. The final experiment in this chapter examined whether the administration of a novel AMPAkine, drug 9a, which enhances the activity of already active AMPA receptors influenced recognition memory.

5.1 Introduction

Glutamate is a major excitatory neurotransmitter in the central nervous system, and the contribution of glutamate to synaptic transmission and plasticity is well established (Nakanishi, 1994). Synaptic glutamate receptors are located primarily on the membranes of neuronal cells, and are responsible for the glutamate mediated postsynaptic excitation of neural cells (Nicoletti et al., 2011). Fast synaptic transmission is mediated by three main classes of ionotropic glutamate receptor; the AMPA receptors, the kainate receptors and NMDA receptors (Nakanishi, 1994). Ionotropic glutamate receptors are ligand-gated cation channels which allow the influx of potassium, sodium and calcium ions in response to glutamate binding. Upon influx of the positive ions, an excitatory post synaptic potential is generated, which causes depolarization in the membrane, and when a threshold is reached, can cause the generation of an action potential in the postsynaptic neuron (Traynelis et al., 2010). This chapter will focus specifically on 'non-NMDA' receptors; the AMPA and kainate receptors. AMPA and kainate receptors are often tetra- or pentameric ion channels which are composed of a number of subunits which form homo- or heteromers. There are four subunits of the AMPA receptor; GluR1, GluR2, GluR3 and GluR4 (Malinow & Malenka, 2002) and five subunits of the kainate receptor; GluK1, GluK2, GluK3, GluK4 and GluK5. However, the number of different subunit compositions which exist to form functional AMPA and kainate receptors is unknown (Paternain, Herrera, Nieto, & Lerma, 2000). This introduction will discuss the role of AMPA receptors in learning and memory as a whole, and the implications that this may have on the behavioural pattern seen in the Tc1 mouse model. The role of kainate receptors in learning and memory as a whole will then be discussed, before focussing more closely on the GluK1 and GluK5 subunits, and their hypothesised impact on the behavioural deficits seen in the Tc1 mouse model.

5.1.1 AMPA Receptors

AMPA receptors are difficult to characterise with regards to their role in learning and memory; agonists are excitotoxic, meaning that over activation of the receptors upon which they act results in damage and possibly neuronal death, and antagonists prevent excitation so heavily that they essentially act as an anaesthetic, temporarily deactivating a brain region (Riedel, Platt, & Micheau, 2003). In addition, AMPA blockade also hampers NMDA activation, and so the effect of AMPA blockade is never exclusively due to reduced AMPA activity (Riedel, Platt and Micheau, 2003). Nevertheless, there is still a substantial body of work on AMPA receptors and their role in learning and memory. The administration of AMPA antagonists essentially results in the inhibition of a brain

region. For example, the AMPA receptor antagonist LY326325 can be administered long term, and infusion directly into the hippocampus during the acquisition stage of a spatial reference memory task in the MWM has been shown to impair learning (Riedel et al., 1999). In addition, administration prior to training impaired learning, and administration prior to a probe trial impaired recall (Riedel et al., 1999). The effect of LY326325 was brain region specific, and also task specific; visual discrimination was not impaired, though this may well be due to the region of infusion as opposed to the task specific role of AMPA receptors; AMPA antagonists reveal more about a regional involvement than they do a specific role of AMPARs. The data are in broad agreement with experiments by Filliat, Pernot-Marino, Baubichon, and Lallement (1998) in which administration of the AMPA antagonist NBQX impaired acquisition of memory during a MWM task. However, this experiment failed to find evidence of disruption to the retention of memory upon blockade of AMPA receptors. An additional study by Liang, Hon, Tyan, and Liao (1994) investigated the effect of administration of the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) into the hippocampus under one of the three schedules: 5 min prior to each daily training session, immediately after each daily training session or 5 min prior to the final testing trial. Pre-training intra-hippocampal infusion of CNQX impaired acquisition, but post-training infusion of CNQX had no effect. However, pre-test infusion markedly impaired retrieval of the already-formed spatial memory. Taken together, these findings suggest a role for hippocampal AMPA receptors, during spatial information processing.

In addition to the impairments on spatial memory tasks as a result of AMPA receptor blockade, there is also evidence to suggest that AMPA receptors have a role in other learning and memory tasks. Accordingly, Winters and Bussey (2005) reported involvement of AMPA receptors in the perirhinal cortex in several stages of object recognition memory. Consistent with a role for AMPA receptors in fast synaptic transmission, Winters and Bussey (2005) showed that intra-perirhinal infusions of the AMPA receptor antagonist CNQX disrupted three stages of object recognition memory in a novel object recognition task. Infusions of CNQX before the sample phase impaired object recognition with short (5 min) and long (3 h) retention delays, suggesting a critical role for perirhinal AMPA receptors in the initial acquisition of the object representation. CNQX infused immediately after the sample phase also impaired object recognition memory when tested with a 3-h retention delay, indicating a role for AMPA receptors in the perirhinal cortex in the storage of the object memory trace (Winters and Bussey, 2005). Finally, intra-perirhinal infusions of CNQX before the choice phase also impaired recognition memory, suggesting the importance of AMPA receptors in the perirhinal cortex at the retrieval stage in the novel object recognition task. Although collectively

data suggest a role for AMPA receptors in learning and memory, there is also the possibility that the behavioural deficits following AMPAR blockade could be caused by non-specific and/or sensorimotor effects of the drug, or the result of secondary NMDA signalling changes, and this must be taken into consideration before making any solid conclusions.

Plasticity at synapses can be regulated at the pre-synaptic site by changing the release of neurotransmitter molecules, such as glutamate, or post-synaptically by changing the number, types, or properties of neurotransmitter receptors, such as AMPA receptors (Kessels & Malinow, 2009). Studies using in vitro synaptic plasticity models have identified the regulated trafficking of post-synaptic AMPA glutamate receptors as a key mechanism underlying activity-induced changes in synaptic transmission. Kessels and Manilow (2009) for example discovered that although many mechanisms can regulate the onset or magnitude of LTP and LTD, in many cases, there appears to be one common mechanism controlling the postsynaptic expression: the addition and removal, respectively, of synaptic AMPA receptors, and this can be seen when learning is taking place. Specifically, the GluR1 subunit of the AMPA receptor has been consistently regarded as a key mediator of hippocampal synaptic plasticity.

In a study by Whitlock, Heynen, Shuler and Bear (2006) in which rats were trained to avoid a fearful context, electrodes positioned in their hippocampus measured enhanced transmission 30 minutes after learning. This increase in transmission was accompanied with increased AMPA receptor phosphorylation. This is suggestive that the learning of a contextual fear conditioning paradigm leads to synaptic AMPA receptor trafficking in hippocampal neurons. It does not, however, provide evidence that LTP-like mechanisms participate in or are required for the modified behaviour. Mice lacking the gene encoding the GluR1 subunit (Zamanillo et al., 1999) however, have demonstrated that while basal AMPA receptor mediated transmission in hippocampal slices were normal, LTP was deficient. This suggests that synaptic trafficking of GluR1-containing AMPA receptors is necessary for the induction of LTP. More specifically, it is especially important for a short-lasting, rapidly induced form of potentiation (Erickson, Maramara, & Lisman, 2010; Romberg et al., 2009).

The behavioural profile of GluR1 knockout mice is theoretically interesting as it suggests differential involvement of these receptors in short and long-term memory processes. Mice lacking GluR1 show normal acquisition of spatial reference memory tasks in which they must discriminate between locations that are either always rewarded or non-rewarded. In contrast, GluR1 knockout mice failed to discriminate between locations during spatial working memory tasks (Schmitt, Deacon, Seeburg, Rawlins, &

Bannerman, 2003). Where normal mice preferentially explore a novel location over a familiar location, GluR1 knockout mice failed to show short-term memory for a familiar spatial environment and consequently showed an equal preference for exploring a novel location and a familiar location (Sanderson et al., 2007). In contrast, GluR1 knockout mice were capable of forming long-term associative memories (Reisel et al., 2002; Schmitt et al., 2003; Zamanillo et al., 1999), a pattern of behaviour which is in line with the idea that short-term and long-term memory depend on dissociable physiological processes (Izquierdo, Medina, Vianna, Izquierdo & Barros, 1999). A study by Sanderson et al. (2009) reported that GluR1 knockout mice showed impaired performance on a y-maze spatial novelty task when the interval between trials was short (1 min), but showed enhanced spatial memory if the interval between the trials was long (24 h). These data firstly provide evidence against a model whereby short-term memories are serially converted into long-term memories, and provide evidence for dissociable memory processes. Secondly, the data indicate a role for GluR1 in short term spatial memory processes.

Sanderson et al. (2011) tested GluR1 knockout mice on a standard object recognition task whereby mice were exposed to two copies of item A, before being tested with a copy of item A, and a novel item, B (AA→AA→AB). In addition, the GluR1 knockout mice were also tested on a recency memory task, where they are exposed to two copies of object A followed by two copies of object B, before receiving a test with A and B (AA→BB→AB). Wagner (1981), provides one interpretation of this; in the test phase, the memory of the more recently presented would be stronger than the memory of the less recently presented object (i.e., A), because it has had less time to decay, thus, mice should show a preference for object A over B. In the object recency task both objects are exposed and tested in the same context. Initially, it appeared that knockout of GluR1 failed to cause a deficit on either the object recognition or recency-dependent tasks. However, on further examination of the data, it became apparent that GluR1 knockout mice displayed increased levels of exploration of the objects in both the sample and test phases compared with the wild type control animals. As a result, the experiments were repeated, and the amount of time the GluR1 knockout animals spent exploring the objects was yoked to that of the control mice during the sample phase. Under yoked conditions, it was found that the GluR1 knockout mice showed impaired performance on both the object recognition and the recency-dependent tasks.

The context-dependent version of the task involved exposure to object A in context X and exposure to object B in in context Y (Sanderson & Bannerman, 2011; Sanderson et al., 2011). In the test trial mice were allowed to explore objects A and B in either context X or Y, and the prior formation of an association between the context and the object

would result in mice showing a preference for exploring the object not previously paired with the context used in the test. Only by learning the association between context and objects could recognition memory be demonstrated in this task. In contrast to the context independent recency version of the task, GluR1 knockout did not impair the performance of mice on a context-dependent recognition task, even when yoked. The results from this experiment suggest a role for the GluR1 AMPA receptor subunit in non-spatial as well as spatial recognition memory, as well as in recency-dependent memory processes. Sanderson et al., (2011) also pointed out that the result suggests that short-term and long-term memory reflect non-associative and associative processes respectively, which is interesting in the light of Wagner's (1981) SOP model.

Previous research has demonstrated reduced surface receptor expression of the GluR1 subunit of the hippocampal AMPA receptor in the Tc1 mouse model of trisomy-21 (Morice et al., 2008). Expression in the perirhinal cortex, however, was not determined. The pattern of short-term memory impairments in Tc1 mice together with evidence of altered GluR1 expression in the hippocampus suggests that expression of these receptors may be altered in the perirhinal cortex (a region recognised for is contribution to object recognition).

5.1.2 Kainate Receptors

Kainate receptor subunits are widely distributed throughout the brain. GluK1-3 subunits are found in somewhat higher levels in the CA3 region of the hippocampus, the striatum and the inner layers of the cortex. In contrast, GluK4 and 5 subunits have a much more restricted distribution, with GluK4 expressed almost exclusively in the hippocampus (Darstein, Petralia, Swanson, Wenthold, & Heinemann, 2003). GluK5 subunits are also found in the striatum and the inner/outer layers of the cortex (Gallyas, Ball, & Molnar, 2003). Activation of kainate receptors regulate glutamate release, and both depress and facilitate transmission in different synapses at different stages of development (Chittajallu et al., 1996). Pre-synaptic kainate receptors play a very significant role in long-term potentiation; LY382884, an antagonist that is selective for GluK1 containing receptors, has been shown to block the induction of NMDA receptor-independent LTP in CA3 hippocampal neurons (Bortolotto et al., 1999), indicating the involvement of GluK1-containing receptors in synaptic plasticity. This has been confirmed by the use of the GluK1 selective antagonist, UBP296 (More et al., 2004). The synaptic activation of pre-synaptic kainate receptors has been shown to lead to the increased release of glutamate, which leads to increased synaptic transmission and thus further increased activation of

pre-synaptic kainate receptors (Lauri et al., 2001). Upon the induction of mossy fibre LTP, there is complete inhibition of kainate receptor-mediated facilitation of synaptic transmission, suggesting that these two processes are linked and it may well be that frequency facilitation via the kainate receptors is the trigger for the induction of LTP.

A major difficulty in the study of the functions of kainate receptors has been the inability to distinguish between individual subunits, and indeed between kainate and AMPA receptors, with pharmacological agents. Developments over the last few years have led to the introduction of new compounds that show a good degree of selectivity. The GluK4-5 subunits (formerly KA1 and KA2) are distinct both structurally and functionally from the GluK1-3 subunits. They are obligate heteromers, and must assemble with GluK1-3 subunits to produce functional surface receptors which contain two of each subunit type (Gallyas et al., 2003; Perrais, Veran, & Mulle, 2010; Reiner, Arant, & Isacoff, 2012). Incorporation of a GluK4 or GluK5 subunit changes the functional and pharmacological properties of recombinant receptors, increasing sensitivity to glutamate, allowing activation by AMPA, slowing deactivation, and altering the concentration-dependence of desensitization (Fisher & Mott, 2011; Mott, Rojas, Fisher, Dingledine, & Benveniste, 2010; Sakimura, Morita, Kushiya, & Mishina, 1992).

Perhaps one of the most interesting aspects of the pattern of behavioural results presented so far in this thesis is that short-term and long-term recognition processes were dissociated in Tc1 mice. One interpretation of this finding is that in Tc1 mice, cortical systems supporting short-term object memory were disrupted. Barker et al., (2006) reported that infusion of a kainate receptor antagonist UBP302 (a selective GluK5 antagonist) into the perirhinal cortex impaired recognition memory following a short (20-min) delay but not following a long (24-h) delay. Antagonism of perirhinal NMDA receptors produced the opposite pattern of results. These results suggest distinct and independent forms of plasticity (Barker et al., 2006). It remains possible that trisomy of Hsa21 genes in Tc1 mice may impact on these cortical receptors. It remains possible that the expression/activity of perirhinal kainite receptors is altered in Tc1 mice, and this chapter presents the results of immunohistochemical experiments which were carried out in order to explore this hypothesis.

5.1.3 Administration of a novel AMPAkine (Drug 9a) and Modafinil

Abnormalities in glutamatergic signalling are thought to underlie a number of debilitating psychiatric diseases. As a result, interventions to modulate this signalling pathway offer considerable promise as potential approaches to treat cognitive impairment (Ward et al., 2011). As has been mentioned previously throughout this chapter, AMPA receptors

mediate the majority of fast excitatory signalling in the central nervous system and thus provide a potential target for the modulation of glutamatergic signalling. However, a lot of previous attempts at modulating AMPA receptor activity has proved difficult; agonists, which are able to act directly on AMPA receptors have been found to be poorly tolerated as they lead to uncontrolled central stimulation, causing seizure activity (Ward et al., 2011). As a result of this negative aspect of AMPA receptor pharmacology, positive modulation of the AMPA receptor has been thought to be more desirable, and thus more heavily focused on recently. More effective activity dependent AMPAkines are starting to be developed, which have no direct effect upon channel currents, and are only able to enhance ion flux through the receptor in the presence of the endogenous ligand glutamate; thus they enhance the activity of already active AMPA receptors. Work by Ward et al. (2011) has as a result of these criteria, uncovered a novel AMPA receptor positive allosteric modulator that binds effectively into the pocket common to all known modulators but with a unique and highly conserved mode of interaction via a triflouromethyl group. Development candidate, drug 9a is a potently efficacious molecule with an attractive safety profile in preclinical species (Wilkinson, personal communication).

Drug 9a has previously been shown to have cognitive enhancing effects in rats performing the novel object recognition task (Wilkinson, personal communication). Given the deficit of the Tc1 mouse on the novel object recognition task following a ten minute retention period, the Tc1 mouse provides a useful model to test whether drug 9a has any cognitive enhancing effects. Together with the data demonstrating that there is reduced GluR1 expression across all sub-regions of the hippocampus (CA1, CA3 and dentate gyrus), and the perirhinal cortex of Tc1 mice, it is hypothesised that the AMPAkine, drug 9a will have a positive allosteric modulating effect on the AMPA receptors that are active within the Tc1 mouse, and thus enhance AMPA receptor activity, thereby improving performance on the novel object recognition task.

Given that drug 9a has a thirty minute lag time, the drug was administered prior to the acquisition phase. Experiment 2a tested whether the Tc1 mice showed a deficit at a forty minute retention period, which would have allowed the drug to be tested after the acquisition phase, and prior to recall. Experiment 2b tested the effects of the novel AMPAkine, drug 9a, on the performance of Tc1 and WT animals on the novel object recognition task. Finally experiment 2c examined the effects of Modafinil on the performance of Tc1 and WT animals on the novel object recognition task. Modafinil is a wakefulness promoting agent which is used for the treatment of sleep disorders such as narcolepsy. However, it is also recognised that Modafinil has general cognitive

enhancing effects; when sixty healthy young adult male volunteers received either a single oral dose of placebo, or 100 mg or 200 mg Modafinil prior to performing a variety of tasks designed to test memory and attention, Modafinil significantly enhanced performance on tests of digit span, visual pattern recognition memory, spatial planning and stop-signal reaction time in a non-dose dependent manner (Turner et al., 2003). Modafinil was initially believed to be a selective, atypical dopamine reuptake inhibitor, however, it appears that other additional mechanisms may also be at play, with studies suggesting that Modafinil effects histamine, serotonin, GABA and glutamate ((Ferraro et al., 2013; Ferraro et al., 1997; Ferraro et al., 1996; Ishizuka, Sakamoto, Sakurai, & Yamatodani, 2003). Thus, testing the effects of Modafinil on the Tc1 and WT mice whilst performing the novel object recognition task provided some information about the nature of the specificity of drug 9a.

5.2 Materials and Methods

5.2.1 Subjects

Male Tc1 mice and their age-matched wild type (WT) male litter mates were bred at the Francis Crick Institute, London, transferred to Cardiff University and housed as described in chapter 3.

Experiment 1 (a, b and c) were carried out using a cohort of 10 male (5 Tc1 and 5 WT) mice. They were housed as described in the general methods section, and were approximately 12 months old at the beginning of the immunohistochemical imaging studies. These animals had previously experienced basic behavioural testing on novel object recognition, object in place and object location tasks (chapter 3).

Experiment 2 (a, and b) were carried out using a cohort of 24 male (12 Tc1 and 12 WT) mice. They were housed as described in the general methods section, and were approximately 10 months old at the time of testing. Experiment 2c was carried out using 23 mice (12 WT and 11 Tc1) due to attrition.

5.2.2 Experiment 1

5.2.2.1 Tissue Preparation

Mice were deeply anaesthetised via intra-peritoneal injection of 0.2ml of Euthatal (sodium pentobarbital). The mice were then exsanguinated, via insertion of a cannula into the left cardiac ventricle, with 80ml of 0.01M phosphate buffered saline (PBS) at pH 7.4. This was followed immediately by perfusion with 100ml of cold 4% paraformaldehyde in 0.01M PBS (PFA). The brain tissue was extracted from the skull and post-fixed for a further 8 hours in 4% PFA at room temperature on a stirrer plate, before being transferred to a solution of 25% sucrose in double distilled water (ddH_2O) for 48 hours at 4°C, until the brain tissue sank to the bottom of the solution. Tissue was mounted on a freezing microtome at -20°C and cut into 40µm coronal sections. A series of one in six sections was collected in PBS. Tissue sections were stored at -20°C in an ethylene-glycol based cryoprotectant until used for immunohistochemical analysis.

5.2.2.2 Receptor Immunohistochemistry

In order to reduce variability, a series from one mouse from each group were processed concurrently. First, sections brought to room temperature and were then washed six times for five minutes in 0.1 M PBS in order to remove cryoprotectant. Sections were then washed once for thirty minutes in Quench, containing methanol (20 % conc), hydrogen peroxide (1.5% conc) and PBS (137 mM NaCl, 1.5mM KH2PO4, 8 mM Ma2HPO4, 2.5 mM KCl, pH 7.4), followed by a further three washes for five minutes in PBS. Sections were then blocked once for thirty minutes in a block solution containing bovine serum albumin (BSA) 1% (w/v), normal goat serum 3% (v/v) (NGS) and triton-x 0/1% (v/v), in order to block endogenous peroxidases. Sections were then incubated in primary antibody solution, diluted in block solution: polyclonal rabbit-anti-GluR1 (1:1000; abcam, cat no. ab31232), polyclonal rabbit-anti-GRIK1 (1:1000, novus, cat no. NB100-91785) and polyclonal rabbit-anti-GRIK5 (1:1000, novus, NBP1-76853) for 24 hours at 4° C on a stirrer plate. Plates were sealed with parafilm to avoid evaporation. Sections were washed three times for five minutes in PBS, before being incubated in secondary antibody solution, biotinylated goat-anti-rabbit (1:250; Vector Laboratories) diluted in block solution for 2 hours at room temperature. Next, sections were washed three times for five minutes in PBS, and incubated in avidin-biotinylated horseradish peroxidase complex in PBS (abc) (VECTASTAIN Elite ABC HRP Kit, Vector Laboratories) for forty five minutes at room temperature. Sections were then washed three times for five minutes in PBS. Finally, diaminobenzidine 0.5 mg/ml (DAB Substrate kit, Vector Laboratories) was used to visualize the reaction. This reaction was then stopped in cold ddH_2O. Sections were mounted onto gelatin-coated slides, dehydrated in an ascending series of alcohol concentrations (5 minutes in 100%, 5 minutes in 95%, 5 minutes in 80%, and 5 minutes in 70% x 2) and cleared in xylene before being cover-slipped using Di-n-butyl phthalate (DPX) mounting medium.

5.2.2.3 Regions of Interest

As described in Chapter 4

5.2.2.4 Image capture and analysis of receptor expression

As described in chapter 4, but for each section the regions of interest were defined by hand, and the number of immunoreactive cells within these regions was determined by converting images to greyscale, and taking a mean grey intensity value; this quantifies the average darkness of the image due to DAB signal. Areas with tears were avoided, to prevent bias by bringing down the average optical density.

5.2.3 Experiment 2

5.2.3.1 Apparatus

As described in Chapter 3

5.2.3.2 Experimental Design

As described in Chapter 3

5.2.3.3 Behavioural Methods

Experiment 2a: Tc1 novel object recognition following a 10-min or 40-min delay

As described in Chapter 3, but using a 10-minute and 40-minute delay interval.

Experiment 2b: Tc1 novel object recognition following a ten minute delay, following the administration of a novel AMPAkine, drug 9a

Performance on a novel object recognition task was tested at baseline, with administration of vehicle, and also under the effects of drug 9a, a novel AMPAkine. Drug 9a (Vehicle, 0.1, 0.3 and 1 mg/kg), was prepared in methylcellulose fresh on the day of use, and administered via oral gavage prior to the first exposure phases (Ward et al., 2011). Treatments were given using a Latin-square design, with at least two days between treatments. The experimenter was blind to both genotype and to the assignment of the animals to each drug condition.

Following drug administration, and a thirty minute lag period, mice were placed in the centre of the arena and presented with three different objects, each in a different corner of the arena. Mice were allowed to explore the arena and the objects for ten minutes before being removed for a 10-min interval spent in their home cage. Mice were then given a second 10-min sample phase before being returned to their home cage for a 10-

min retention interval. In the test phase, one of the items was replaced with a novel object. The time mice spent exploring the novel object and the familiar objects was recorded. The location of the objects and the object that was replaced with the novel item was fully counterbalanced both within and between groups. All objects (novel and familiar) and the arena were wiped down with a 5% alcohol/distilled water wipes between sample phases and prior to returning the mouse to the apparatus for the test stage. Discrimination ratios were calculated as follows: time exploring the novel object/ (time spent exploring the novel object + (average time exploring both familiar objects)).

Experiment 2c: Tc1 novel object recognition following a ten minute delay, following the administration of Modafinil

As described in experiment 2b, but Modafinil was administered (32mg/kg, 64mg/kg). Vehicle data from experiment 2b was used due to time restrictions. Modafinil was prepared in physiological saline fresh on the day of use, and administered via intraperitoneal injection prior to the first exposure phases (Fernandes et al., 2013). Treatments were given using a Latin-square design, with at least two days between treatments.

5.3 Results

5.3.1 Experiment 1a: GluR1 Expression

The number of immunoreactive cells in the hippocampus (CA1, CA3 and Dentate Gyrus) and the perirhinal cortex was determined by converting images to greyscale, and taking a mean grey intensity value; this quantifies the average darkness of the image due to DAB signalling. The mean grey intensity value for the regions of the hippocampus can be seen in figure 5.1 (a) and the mean grey intensity value for the perirhinal cortex can be seen in figure 5.1 (b).

A repeated measures ANOVA with ROI; CA1, CA3 and DG, as the within subjects factor, and genotype as between subjects factor revealed a significant main effect of ROI ($F_{(1, 8)} = 5.877$, $p = 0.012$), but no significant main effect of genotype ($F_{(1, 8)} = 1.341$, $p = 0.280$) The interaction between ROI and genotype was, however, significant ($F_{(1, 8)} = 20.023$, $p = 0.001$). Tests of simple effects revealed a significant effect of genotype in the DG ($F_{(1, 8)} = 6.903$, $p = 0.030$), the CA1 ($F_{(1, 8)} = 6.760$, $p = 0.032$) and the CA3 ($F_{(1, 8)} = 7.051$, $p = 0.029$). There was also a significant effect of ROI on both WT animals ($F_{(1, 8)} = 15.304$, $p = 0.003$), and on Tc1 animals ($F_{(1, 8)} = 11.218$, $p = 0.007$). An example of GluR1 staining in the hippocampus can be seen in figure 5.2 (a).

Furthermore, an independent samples t-test revealed that the mean grey intensity values for GluR1 receptor expression in the perirhinal cortex of the Tc1 mice were not significantly different to WT mice ($t_{(8)} = 1.992$, $p = 0.082$). An example of GluR1 staining in the perirhinal cortex can be seen in figure 5.2 (b).

In summary, these results confirmed reduced GluR1 receptor subunit expression in the dentate gyrus of Tc1 animals, and are novel in that they demonstrate differences in GluR1 receptor expression in the CA1 and CA3 regions of the hippocampus of Tc1 mice. The data also suggest a trend towards a decrease in GluR1 expression in the perirhinal cortex of Tc1, although the results did not reach statistical significance.

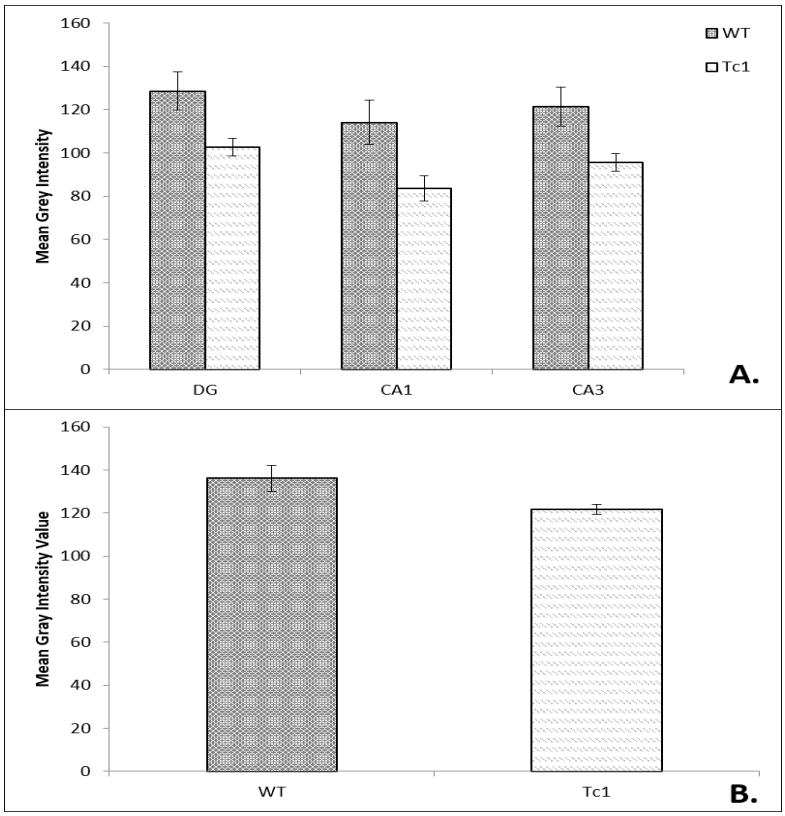


Figure 5.1-Mean grey intensity values for GluR1 expression *in the a) hippocampus and b) perirhinal cortex of Tc1 and WT control mice.*

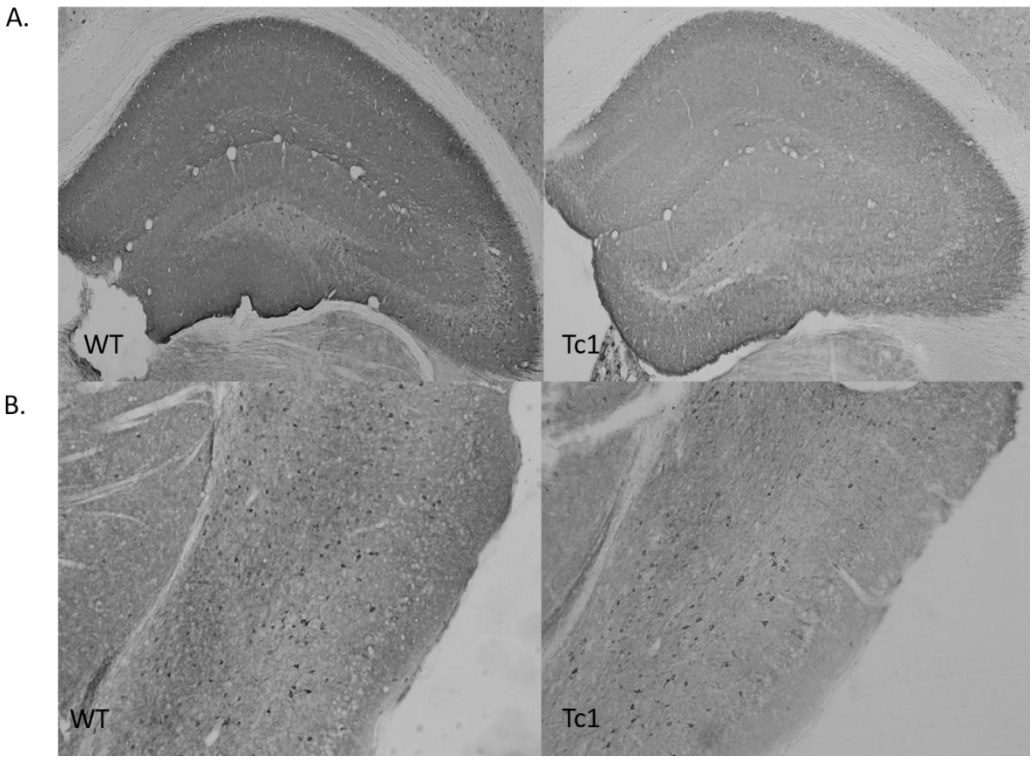


Figure 5.2 – Example images of GluR1 expression *in the a) hippocampus and b) perirhinal cortex of Tc1 and WT control mice.*

5.3.2 Experiment 1b: GluK1 Expression

The number of immunoreactive cells in the hippocampus (CA1, CA3 and Dentate Gyrus) and the perirhinal cortex was determined by converting images to greyscale, and taking a mean grey intensity value; this quantifies the average darkness of the image due to DAB signal. The mean grey intensity value for the regions of the hippocampus can be seen in figure 5.3 (a) and the mean grey intensity value for the perirhinal cortex can be seen in figure 5.3 (b).

A repeated measures ANOVA with ROI; CA1, CA3 and DG, as the within subjects factor, and genotype as between subjects factor revealed no significant main effect of ROI ($F_{(1, 8)} = 2.396$, $p = 0.161$), and no significant main effect of genotype ($F_{(1, 8)} = 0.419$, $p = 0.536$). The interaction between ROI and genotype also failed to reach significance ($F_{(1, 8)} = 0.873$, $p = 0.437$). An example of GluK1 staining in the hippocampus can be seen in figure 5.4 (a).

Furthermore, an independent samples t-test revealed that the mean grey intensity values for GluK1 receptor expression in the perirhinal cortex of the Tc1 mice were not significantly different to WT mice ($t_{(8)} = 0.415$, $p = 0.689$). An example of GluK1 staining in the perirhinal cortex can be seen in figure 5.4 (b). The results suggest no variation in GluK1 receptor subunit expression in the hippocampus or perirhinal cortex of Tc1 mice in comparison to WT mice.

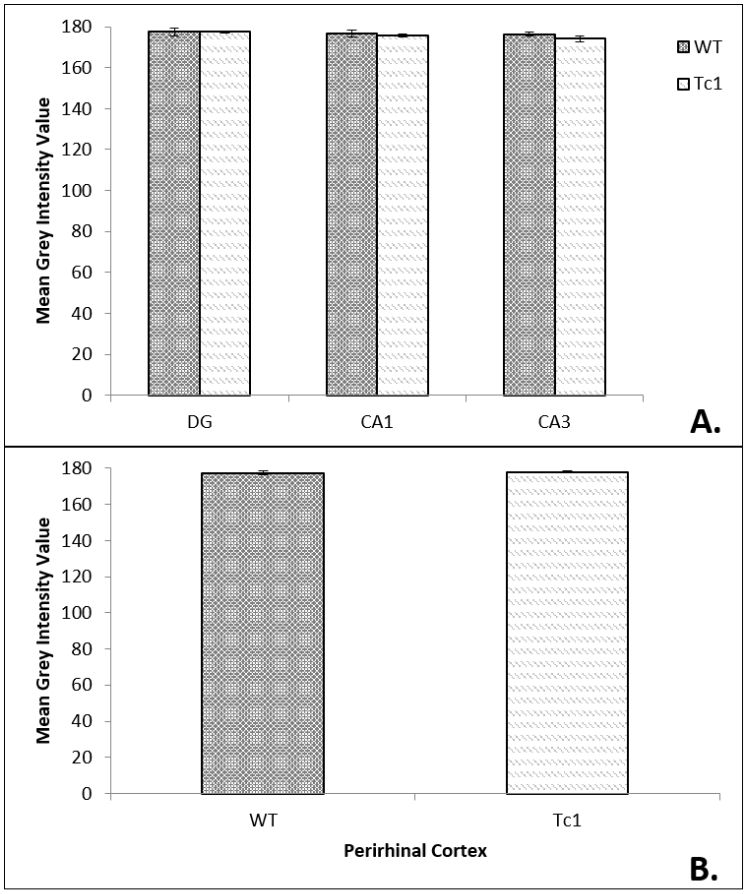


Figure 5.3 - Mean grey intensity values for GluK1 expression *in the a) hippocampus and b) perirhinal cortex of Tc1 and WT control mice.*

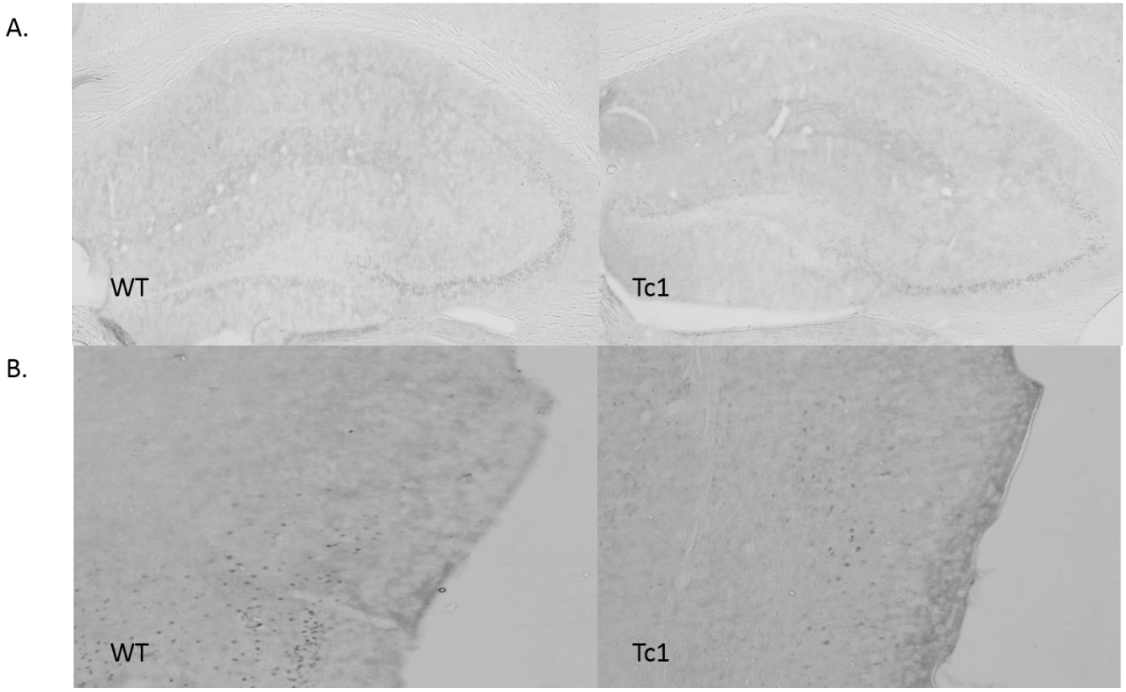


Figure 5.4 - Example images of GluK1 expression *in the a) hippocampus and b) perirhinal cortex of Tc1 and WT control mice.*

5.3.3 Experiment 1c: GluK5 Expression

The mean grey intensity value for the regions of the hippocampus can be seen in figure 5.5 (a) and the mean grey intensity value for the perirhinal cortex can be seen in figure 5.5 (b).

A repeated measures ANOVA with ROI; CA1, CA3 and DG, as the within subjects factor, and genotype as between subjects factor revealed no significant main effect of ROI ($F_{(1, 8)} = 0.262$, $p = 0.754$), and no significant main effect of genotype ($F_{(1, 8)} = 0.087$, $p = 0.776$). The interaction between ROI and genotype failed to reach significance ($F_{(1, 8)} = 2.231$, $p = 0.145$). An example of GluK5 staining in the hippocampus can be seen in figure 5.6 (a).

An independent samples t-test revealed that the mean grey intensity values for GluK5 receptor expression in the perirhinal cortex of the Tc1 mice was significantly reduced in comparison to WT mice ($t_{(8)} = 2.879$, $p = 0.021$). An example of GluK5 staining in the perirhinal cortex can be seen in figure 5.6 (b). These data suggest a specific decrease in GluK5 (KA2) receptor subunit expression in the perirhinal cortex of Tc1 mice when compared to WT mice, but no changes in expression levels in the hippocampus.

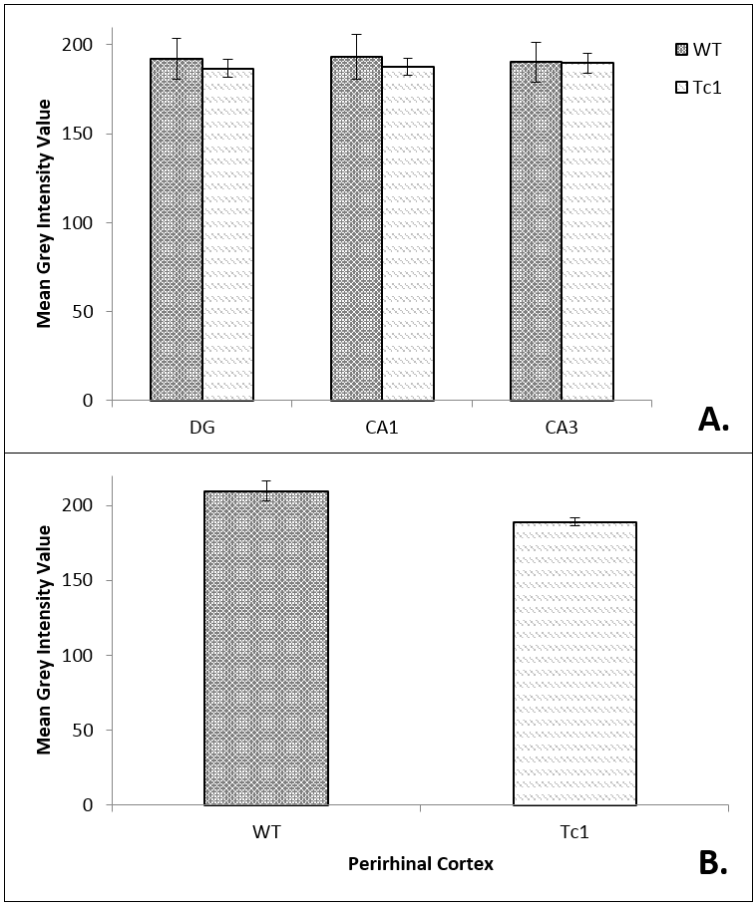


Figure 5.5 - Mean grey intensity values for GluK5 expression *in the a) hippocampus and b) perirhinal cortex of Tc1 and WT control mice.*

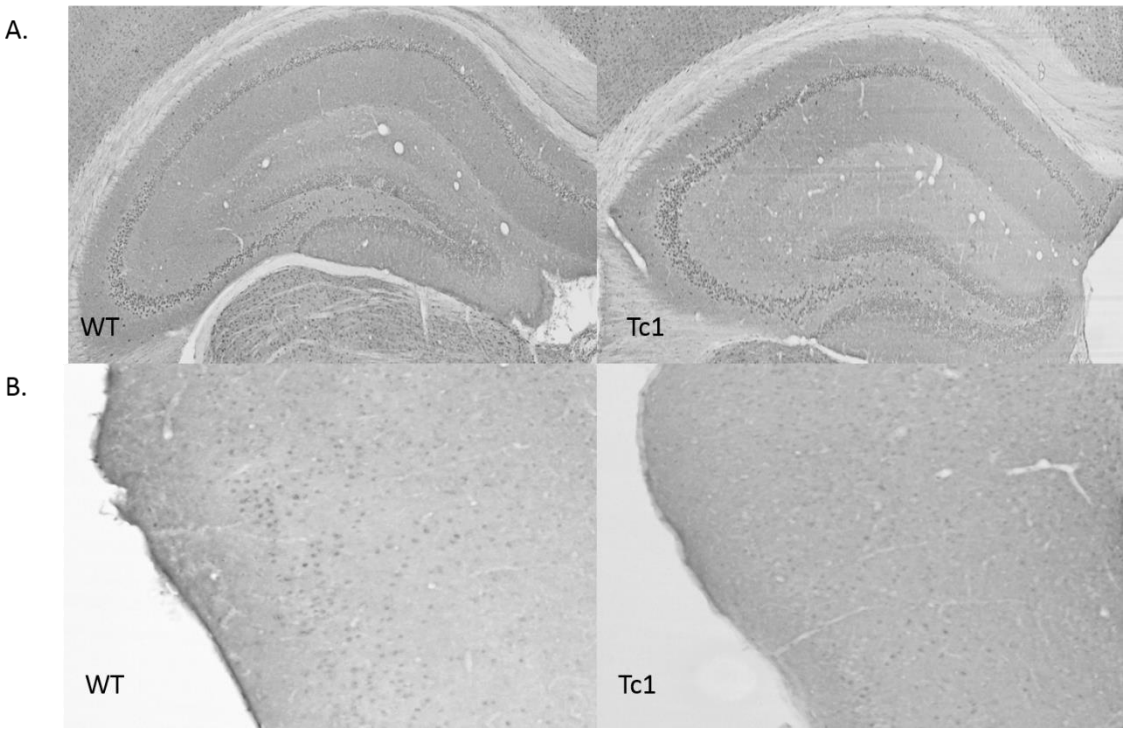


Figure 5.6 - Example images of GluK5 expression *in the a) hippocampus and b) perirhinal cortex of Tc1 and WT control mice.*

5.3.4 Experiment 2a

The main aim of this experiment was to establish whether the Tc1 line was suitable for the pharmacological design described in the current study. Drug 9a has a 30 minute lag time. If the Tc1 mice were to show a deficit on the novel object recognition task following forty minutes, then it would allow for testing the effects of drug 9a at both the acquisition and the retrieval phase. The contact times for each group of mice during the sample phases (collapsed across retention interval conditions) are shown in Table 5.1 and contact times during the test phase in Table 5.2, respectively. Inspection of Table 5.1 suggests that Tc1 mice showed generally numerically higher contact times with the objects than WT mice. However, the duration of contact decreased in both Tc1 and WT at a similar rate across the sample phases. An Analysis of variance (ANOVA) with sample phase as the within subjects factor, and genotype as the between subjects factor revealed a significant main effect of sample phase on object contact time ($F_{(1, 22)}$ = 57.547, p = 0.001), but no significant main effect of genotype ($F_{(1, 22)}$ = 2.349, p = 0.140), and no significant interaction between these variables (F < 1, p = 0.578). This shows that both groups showed a significant decrease in activity (habituation) from sample phase 1 to sample phase 2.

Table 5.2 shows the mean contact times with objects (novel and familiar) across the delay conditions for Tc1 and WT mice. A repeated measures ANOVA using object and delay as the within subjects factors, and genotype as the between subjects factor revealed a significant main effect of object ($F_{(1, 22)}$ = 126.980, p = 0.001) but no significant main effect of delay ($F_{(1, 22)}$ = 1.425, p = 0.245), and no significant main effect of genotype (F < 1, p = 0.357). The interaction between object and genotype, failed to reach statistical significance ($F_{(1, 22)}$ = 2.893, p = 0.103). There was no significant interaction between delay and genotype ($F_{(1, 22)}$ = 3.762, p = 0.065). There was a significant interaction between object and delay ($F_{(1, 22)}$ = 5.760, p = 0.025). Tests of simple effects revealed a significant effect of object at both the ten ($F_{(1, 22)}$ = 109.002, p = 0.001) and the forty minute ($F_{(1, 22)}$ = 65.914, p = 0.001) delay point, and a significant effect of the delay on interaction with familiar ($F_{(1, 22)}$ = 15.062, p = 0.001), but not the novel object ($F_{(1, 22)}$ = 0.095, p = 0.761). There was also a significant three-way interaction between object, delay and genotype ($F_{(1, 22)}$ = 14.296, p = 0.001). Tests of simple main effects revealed a significant effect of genotype on contact with the novel object at the ten minute time point ($F_{(1, 22)}$ = 4.857, p = 0.038), but not at the forty minute time point ($F_{(1, 22)}$ = 2.081, p = 0.163). There was a significant main effect of genotype on contact with the familiar object at both the ten minute ($F_{(1, 22)}$ = 5.859, p = 0.024) and the forty minute time point ($F_{(1, 22)}$ = 6.300, p = 0.020). Tests of simple main effects revealed a significant effect of

object on Tc1 mice at both the ten minute ($F_{(1, 22)} = 11.679$, $p = 0.002$) and forty minute time point ($F_{(1, 22)} = 41.035$, $p = 0.001$), and on WT mice at both the ten minute ($F_{(1, 22)} = 128.764$, $p = 0.001$) and forty minute time point ($F_{(1, 22)} = 25.764$, $p = 0.001$). Tests of simple main effects also revealed a significant effect of delay period on contact with both the novel ($F_{(1, 22)} = 5.266$, $p = 0.032$) and the familiar object ($F_{(1, 22)} = 11.635$, $p = 0.003$) in Tc1 mice, and the familiar ($F_{(1, 22)} = 4.316$, $p = 0.050$) but not the novel object ($F_{(1, 22)} = 3.485$, $p = 0.076$) in WT mice.

In order to evaluate performance that was independent of individual differences in contact times, the data were also analysed using a discrimination ratio and are shown in Figure 5.7. Inspection of this figure indicates that wild type control mice discriminated between novel and familiar objects following both a 10-min and 40-min delay. In contrast, the Tc1 mice successfully discriminated between novel and familiar objects only following the 40-min delay. A repeated measures ANOVA using discrimination ratios as the within subjects factor and genotype as the between subject factor revealed a significant main effect of genotype ($F_{(1, 22)} = 15.644$, $p = 0.001$), and a significant main effect of delay ($F_{(1, 22)} = 7.782$, $p = 0.010$). There was also an interaction between these two factors ($F_{(1, 22)} = 7.766$, $p = 0.010$). Tests of simple main effects revealed a significant effect of genotype at the 10-min delay ($F_{(1, 22)} = 38.621$, $p = 0.001$), but not at the 40-min delay ($F < 1$, $p = 0.369$). Furthermore, one sample t-test confirmed that the performance of the WT mice were significantly above chance at both delays (10 min: $t_{(10)} = 8.862$, $p = 0.001$; 40-min: $t_{(10)} = 5.75$, $p = 0.001$). However, the performance of Tc1 mice was not above chance at the 10 min delay, ($t < 1$), but was above chance at the 40 min delay ($t_{(10)} = 7.631$, $p = 0.001$). These results therefore confirm that Tc1 mice showed impaired 10-min but intact 40-min object recognition memory.

Genotype	Mean contact time during the sample phases (seconds) with all objects for Tc1 and WT control mice									
	Sample Phase 1	Sample Phase 2								
Experiment 2a: Novel object recognition – 10 min vs 40 min delay										
Tc1	35.33	27.48								
WT	30.88	26.19								
Experiment 2b: Novel object recognition – with AMPAkine										
	Vehicle	0.1mg/kg	0.3mg/kg	1mg/kg	Vehicle	0.1mg/kg	0.3mg/kg	1mg/kg		
Tc1	30.40	33.21	30.87	32.75	24.88	21.36	23.51	25.47		
WT	25.10	26.57	22.97	21.50	18.99	17.41	17.96	15.99		
Experiment 2c: Novel object recognition – with Modafanil										
	Vehicle	32mg/kg	64mg/kg		Vehicle	32mg/kg	64mg/kg			
Tc1	30.40	27.93	26.76		24.88	23.66	24.13			
WT	25.10	19.96	23.67		18.99	16.37	20.63			

Table 5.1 – Mean contact time during the sample phases (seconds) with all objects for Tc1 and WT control mice.

Genotype	Mean contact time during the test phase (seconds) with all objects for Tc1 and WT control mice											
	Experiment 2a: Novel object recognition – 10 min vs 40 min delay											
	Novel (10 min)		Novel (40 min)		Familiar (10 min)		Familiar (40 min)					
Tc1	14.26		19.34		10.79		7.25					
WT	18.88		14.76		7.33		5.15					
	Experiment 2b: Novel object recognition – with AMPAkine											
	Novel (Vehicle)	Novel (0.1mg/kg)	Novel (0.3mg/kg)	Novel (1mg/kg)	Familiar (Vehicle)	Familiar (0.1mg/kg)	Familiar (0.3mg/kg)	Familiar (1mg/kg)				
Tc1	12.49	11.47	11.93	12.85	9.95	6.64	5.71	5.68				
WT	8.83	12.01	10.63	11.38	4.15	5.45	4.45	5.11				
	Experiment 2c: Novel object recognition – with Modafanil											
	Novel (Vehicle)	Novel (32mg/kg)	Novel (64mg/kg)	Familiar (Vehicle)	Familiar (32mg/kg)	Familiar (64mg/kg)						
Tc1	12.49	9.82	10.29	9.95	8.03	7.77						
WT	8.83	8.81	11.48	4.15	4.22	5.42						

Table 5.2 – Mean contact time during the test phase (seconds) with novel and familiar objects for Tc1 and WT control mice.

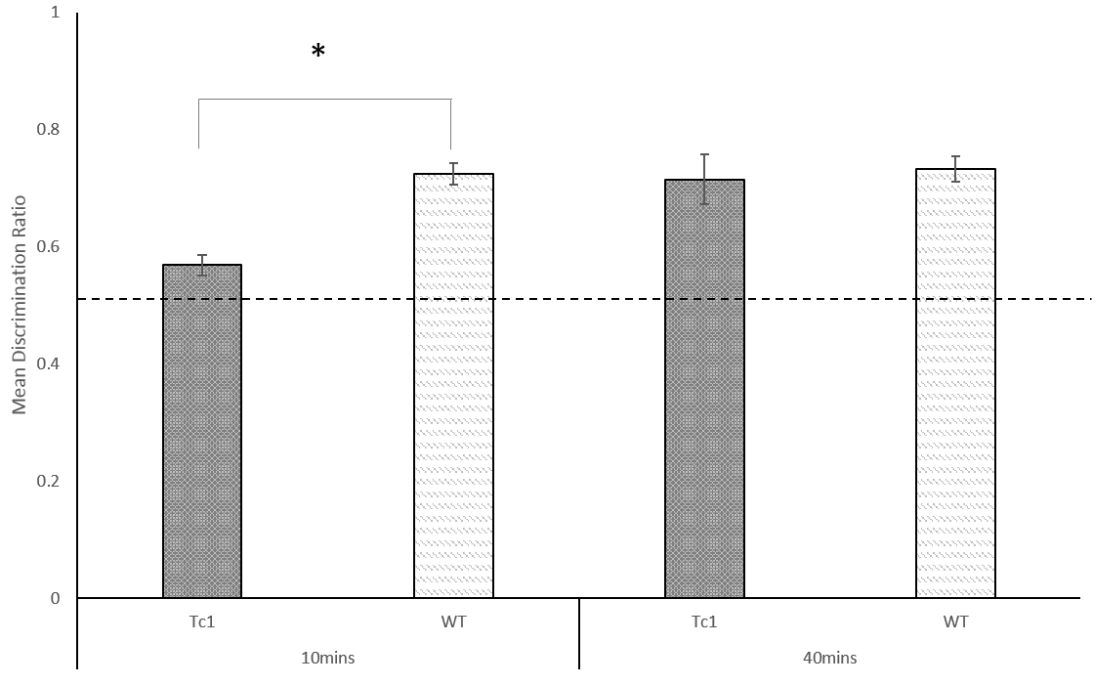


Figure 5.7 – Results of experiment 2a: *Mean discrimination ratio scores for the novel object recognition task following a ten minute or a forty minute delay, in Tc1 and WT control mice. (* = p<0.05, ----------= 0.5, chance).*

5.3.5 Experiment 2b

The main aim of this experiment was to test the effects of a novel AMPAkine, drug 9a, on the performance of Tc1 and WT animals on a novel object recognition memory task, where Tc1 mice normally show a short term deficit. Experiment 2a demonstrated that Tc1 mice show a deficit on a novel object recognition task following a ten minute delay, but not following a forty minute delay. Therefore, due to the 30 minute lag time of drug 9a, the effect of the AMPAkine during the acquisition phase of a novel object recognition task could be tested by administering the drug 30 minutes prior to the sample phases, and then examining the performance of the Tc1 mice following a 10 minute delay to see if there was any improvement in performance. The contact times for each group of mice at each dose during the sample phases are shown in Table 5.1 and contact times during the test phase in Table 5.2, respectively.

Inspection of Table 5.1 suggests that Tc1 mice showed generally numerically higher contact times with the objects than WT mice. However, the duration of contact decreased in both Tc1 and WT at a similar rate across the sample phases. An Analysis of variance (ANOVA) with sample phase and dose as the within subjects factor, and genotype as the between subjects factor revealed a significant main effect of sample phase on object contact time ($F_{(1, 22)} = 62.602$, $p = 0.001$), and a significant main effect of genotype ($F_{(1, 22)} = 5.696$, $p = 0.026$). There was no significant interaction between these variables ($F < 1$, $p = 0.403$). There was no significant main effect of dose ($F_{(1, 20)} <1$, $p = 0.886$). There was no significant interaction between dose and genotype ($F_{(1, 20)} <1$, $p = 0.414$), or between dose and sample phase ($F_{(1, 20)} =2.033$, $p = 0.142$). There was also no three way interaction between sample phase, dose and genotype ($F_{(1, 20)} <1$, $p = 0.908$). This shows that although the Tc1 mice interacted with the objects more than the WT animals, both groups showed a significant decrease in activity (habituation) from sample phase 1 to sample phase 2, and that the drug dose did not significantly alter activity of either genotype during the sample phases.

Table 5.2 shows the mean contact times with objects in the test phase (novel and familiar) across the drug dose conditions for Tc1 and WT mice. A repeated measures ANOVA using object and dose as the within subjects factors, and genotype as the between subjects factor revealed a significant main effect of object ($F_{(1, 22)} = 75.929$, $p = 0.001$) but no significant main effect of dose ($F_{(1, 22)} <1$, $p = 0.573$), and no significant main effect of genotype ($F = 3.097$, $p = 0.092$). The interaction between object and genotype, failed to reach statistical significance ($F_{(1, 22)} <1$, $p = 0.573$). There was no significant interaction between object and dose ($F_{(1, 22)} = 1.410$, $p = 0.269$). There was no significant interaction between dose and genotype ($F_{(1, 22)} = <1$, $p = 0.878$). There

was also no significant three-way interaction between object, dose and genotype ($F_{(1, 22)} = 0.532$, $p = 0.665$).

In order to evaluate performance that was independent of individual differences in contact times, the data were also analysed using a discrimination ratio and are shown in Figure 5.8. Inspection of this figure indicates that wild type control mice discriminated between novel and familiar objects following a 10-min delay when all four drug doses were used. In contrast, the Tc1 mice failed to discriminate between novel and familiar objects following a 10-min delay when the vehicle dose was used, but this performance appeared to improve when the drug was administered in doses of 0.1, 0.3 and 1mg/kg. Because the discrimination ratio data violate the assumption of normality (Shapiro-Wilks test $p<0.05$), a non-parametric statistical test was used. A Kruskal-Wallis H test was run to determine if there were differences in discrimination ratio scores between the two groups of animals; WT and Tc1, with each drug dose. Distributions of discrimination ratio scores were similar for all groups, as assessed by visual inspection of a boxplot. Median discrimination ratio scores were statistically significantly different between WT and Tc1 mice when they were given vehicle, (χ^2 (1) = 5.880, $p = 0.015$). However, the median discrimination ratio scores were not statistically significantly different between WT and Tc1 mice when the drug was delivered, at all three doses (0.1mg/kg, χ^2 (1) = 1.080, $p = 0.299$, 0.3 mg/kg χ^2 (1) = 0.030, $p = 0.862$ and 1mg/kg χ^2 (1) = 0.030, $p = 0.862$). Furthermore, one sample t-test confirmed that the performance of the WT mice were significantly above chance at all doses (Vehicle: $t_{(10)} = 8.396$, $p = 0.001$; 0.1mg/kg: $t_{(10)} = 5.75$, $p = 0.001$, 0.3mg/kg: $t_{(10)} = 6.198$, $p = 0.001$, 1mg/kg: $t_{(10)} = 7.011$, $p = 0.001$). However, the performance of Tc1 mice was not above chance when the vehicle was administered: ($t < 1$), but was above chance level when the AMPAkine was administered at all doses ($t_{(10)} = 8.396$, $p = 0.001$; 32mg/kg: $t_{(10)} = 5.75$, $p = 0.001$, 64mg/kg: $t_{(10)} = 6.198$, $p = 0.001$). This suggests a deficit on the novel object recognition task in Tc1 mice following vehicle administration, but improved performance to the level of WT animals when drug 9a was administered at all three doses.

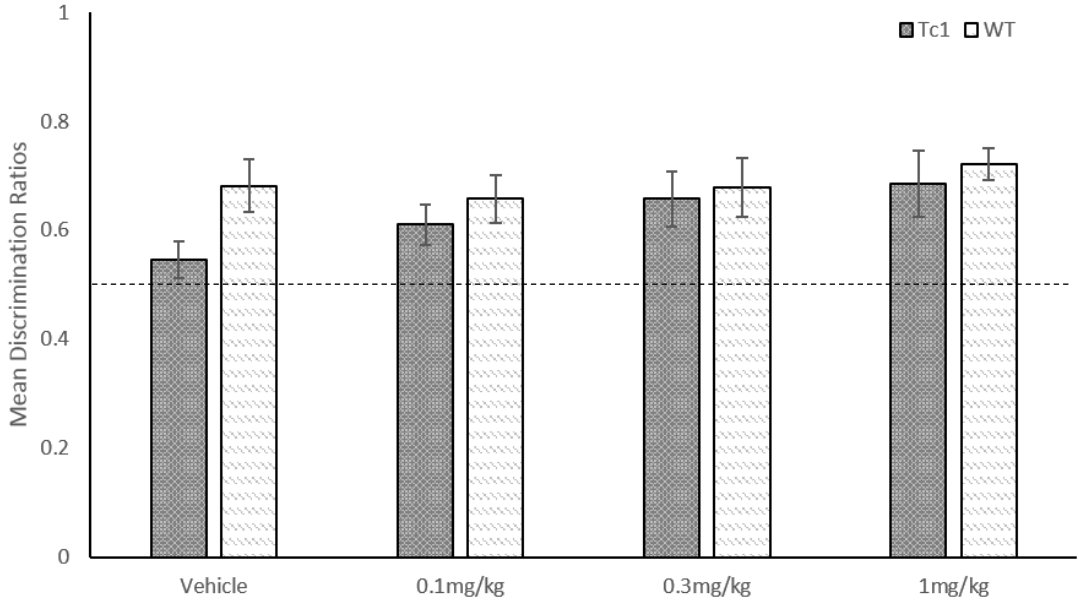


Figure 5.8 – Results of experiment 2b: *Mean discrimination ratio scores on a novel object recognition task following a ten minute delay in Tc1 and WT control mice at vehicle, 0.1 mg/kg, and 0.3mg/kg and 1mg/kg doses of drug 9a. (-------= 0.5, chance).*

5.3.6 Experiment 2c

The main aim of this experiment was to test the effects of Modafinil on the performance of Tc1 and WT animals on the novel object recognition memory task, where Tc1 mice show a short term memory deficit. The contact times for each group of mice during the sample phases (collapsed across retention interval conditions and drug doses) are shown in Table 5.1 and contact times during the test phase in Table 5.2, respectively. An Analysis of variance (ANOVA) with sample phase as the within subjects factor, and genotype as the between subjects factor revealed a significant main effect of sample phase on object contact time ($F_{(1, 21)} = 33.194$, $p = 0.001$), but no significant main effect of genotype ($F_{(1, 21)} = 3.919$, $p = 0.071$). There was no significant interaction between these variables ($F < 1$, $p = 0.909$). There was no significant main effect of dose ($F_{(1, 20)} = 1.317$, $p = 0.290$). There was no significant interaction between dose and genotype ($F_{(1, 20)} < 1$, $p = 0.630$), or between dose and sample phase ($F_{(1, 20)} = 1.422$, $p = 0.265$). There was also no three way interaction between sample phase, dose and genotype ($F_{(1, 20)} < 1$, $p = 0.880$).This shows that both groups showed a significant decrease in activity (habituation) from sample phase 1 to sample phase 2, and that the drug dose did not significantly alter activity of either genotype during the sample phases.

Table 5.2 shows the mean contact times with objects (novel and familiar) across the drug dose conditions for Tc1 and WT mice. A repeated measures ANOVA using object and dose as the within subjects factors, and genotype as the between subjects factor revealed a significant main effect of object ($F_{(1, 21)} = 79.737$, $p = 0.001$) but no significant main effect of dose ($F_{(1, 21)} < 1$, $p = 0.496$). There was a significant main effect of genotype ($F = 7.213$, $p = 0.014$). The interaction between object and genotype was significant ($F_{(1, 21)} = 10.785$, $p = 0.5004$). Tests of simple effects revealed a significant effect of genotype on contact with the familiar object ($F_{(1, 21)} = 19.271$, $p = 0.001$), but not the novel object ($F_{(1, 21)} = 1.153$, $p = 0.295$). Tests of simple effects also revealed a significant effect of object on both WT ($F_{(1, 21)} = 77.975$, $p = 0.001$) and Tc1 ($F_{(1, 21)} = 15.272$, $p = 0.001$) animals. There was no significant interaction between object and dose ($F_{(1, 21)} = 0.254$, $p = 0.777$). There was no significant interaction between dose and genotype ($F_{(1, 21)} = 2.363$, $p = 0.107$). There was also no significant three-way interaction between object, dose and genotype ($F_{(1, 21)} = 0.142$, $p = 0.868$).

In order to evaluate performance that was independent of individual differences in contact times, the data were also analysed using a discrimination ratio and are shown in Figure 5.9. Inspection of this figure indicates that wild type control mice discriminated between novel and familiar objects when all doses of the drug were administered. In contrast, the Tc1 mice were unable to discriminate between novel and familiar objects.

A repeated measures ANOVA using discrimination ratios as the within subjects factor and genotype as the between subject factor revealed a significant main effect of genotype ($F_{(1, 21)} = 4.877$, $p = 0.038$), but no significant main effect of dose ($F_{(1, 21)} < 1$, $p = 0.841$). There was no significant interaction between these two factors ($F_{(1, 21)} <1$, $p = 0.953$). These results confirm that Tc1 mice showed impaired performance on the novel object recognition task following administration of Modafinil at all doses. Furthermore, one sample t-test confirmed that the performance of the WT mice were significantly above chance at all doses (Vehicle: $t_{(10)} = 8.396$, $p = 0.001$; 32mg/kg: $t_{(10)} = 5.75$, $p = 0.001$, 64mg/kg: $t_{(10)} = 6.198$, $p = 0.001$). However, the performance of Tc1 mice was not above chance at any dose, (vehicle: $t < 1$. 32mg/kg, $t < 1$, 64mg/kg: $t < 1$). These results therefore confirm that Tc1 mice showed impaired performance on the novel object recognition task at 10-min despite administration of Modafinil.

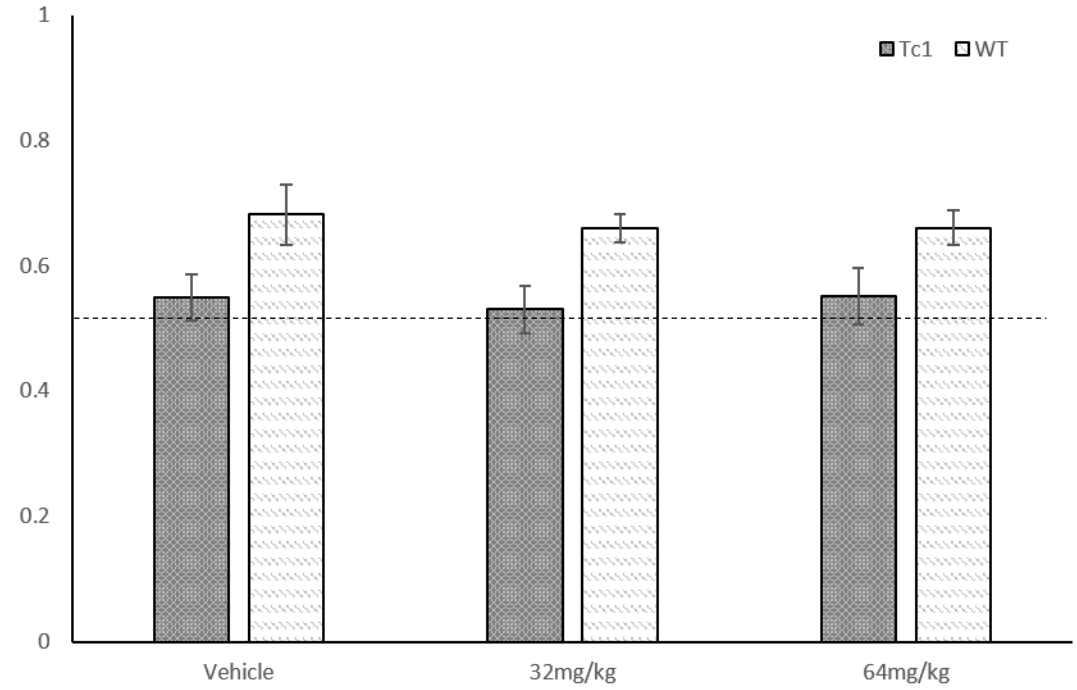


Figure 5.9 –Results of experiment 2c: *Mean discrimination ratio scores on a novel object recognition task following a ten minute delay in Tc1 and WT control mice at vehicle, 32 mg/kg, and 64/kg doses of Modafinil. (---------- = 0.5, chance).*

5.4 Discussion

Previous experiments with Tc1 mice have demonstrated reduced surface expression of the GluR1 subunit of the AMPA receptor in the dentate gyrus of the hippocampus in the Tc1 mouse model of trisomy-21 (Morice et al., 2008). The present study supported previous findings, demonstrating a significant reduction of overall GluR1 subunit expression in the dentate gyrus of the hippocampus, as well as the CA1 and CA3 regions in Tc1 mice. The current study failed to demonstrate a significant reduction in expression of the GluR1 subunit of the AMPA receptor in the perirhinal cortex of Tc1 mice compared with WT controls, however there was a trend towards a difference. The GluK1 subunit of the kainate receptor showed no significant alteration of expression patterns in any region of the hippocampus or the perirhinal cortex. The GluK5 subunit of the kainate receptor however, showed an unaltered expression profile in the hippocampus, but a significant reduction of expression in the perirhinal cortex of Tc1 mice compared with WT control animals. In addition, the administration of a novel AMPAkine improved the performance of Tc1 mice on the NOR task at a 10 minute time point, suggesting perhaps that the AMPAkine administered was able to enhance the activity of already active AMPA receptors in the Tc1 mice, potentially compensating for a reduction in GluR1 expression in these mice.

Previous chapters have demonstrated a short term (10 min) impairment in recognition memory in Tc1 mice, despite long term recognition memory remaining intact. The experiments in this chapter extended this pattern of results and showed that Tc1 mice displayed normal object recognition following a 40-min delay. Thus, object memory remains intact at 40min and 24hr after the sample trial, but not after 10 min. Furthermore, drug 9a, a novel positive allosteric modulator of the AMPA receptor, rescued the deficit in Tc1 mice following a 10 min delay. The specificity of drug 9a in Tc1 mice was confirmed by testing the effects of Modafinil. Modafinil did not influence short-term recognition memory in Tc1 mice.

The fact that Tc1 mice have demonstrate a reduction in the GluR1 subunit of the AMPA receptor in the hippocampus has major implications. Firstly, there is significant evidence that Tc1 mice display aberrant hippocampal short-term, synaptic plasticity, abnormal hippocampal spine morphology, and sub-region changes in the connectivity of the DG-CA3 network that contributes to disruption of place-cell activity (O'Doherty et al., 2005; Witton et al., 2015). Mice lacking the gene encoding the GluR1 subunit display deficient LTP (Zamanillo et al., 1999), which suggests that synaptic trafficking of GluR1-containing AMPA receptors is necessary for the induction of LTP. It is possible that the reduction of

GluR1 expression in the hippocampus of mice is linked to the putative alterations in LTP. However, the hippocampal LTP data, together with a reduction in GluR1 in the hippocampus would suggest that memory for the spatial organisation of objects would be disrupted in Tc1 mice (Burke et al., 2011; Lenck-Santini, Rivard, Muller, & Poucet, 2005) a hypothesis which was not supported by the results from this thesis.

GluR1 knockout mice show a very distinct dissociation between different types of spatial memory. Mice lacking GluR1 showed normal acquisition of spatial reference memory tasks; for example when tested on a reference version of the MWM whereby learning is dependent on the formation of associations between the platform location and extramaze cues, GluR1 knockout mice performed at a comparable level to control mice (Reisel et al., 2002). It is interesting to note, however, that this is a very different phenotype compared to a complete blockade of fast synaptic transmission with an AMPA receptor antagonist; administration of LY326325 results in a deficit in spatial reference memory in the MWM (Reisel et al., 2002). The difference between a lack of GluR1 subunit expression, and complete loss of AMPA receptor mediated transmission is important to acknowledge. Thus it is a possibility that the maintenance of some spatial memory processing ability in the Tc1 mouse, may be a result of compensatory mechanisms from other AMPA receptor subunits. In contrast, a T-maze rewarded alternation task, which is a test of spatial working memory and relies on the ability to remember unique information from a single trial, revealed a deficit in GluR1 knockout mice (Reisel et al., 2002). This observation suggests a spatial “working memory” task, as defined by Olton and Papas (1979), may reveal a deficit in Tc1 mice (see Witton et al., 2015).

Of course, the results of the present study demonstrate a reduction in GluR1 in the hippocampus of Tc1 mice, but no significant reduction in the perirhinal cortex. However, there is a trend towards a reduction of GluR1 in the perirhinal cortex, which also speaks to data that suggests that GluR1 receptors are necessary for object recognition memory. GluR1 knockout mice have been shown to display impaired performance on the object recognition task (Sanderson et al., 2011). The significant reduction of GluR1 expression in the hippocampus of the Tc1 mouse may be substantial enough therefore to disrupt object memory.

Finally, the reduction in GluR1 in the hippocampus of Tc1 mice provides a potential mechanistic explanation for the deficit in short term (10 min) but intact long term (24 hour) recognition memory. When GluR1 knockout mice were tested on both short-term and long-term spatial memory using a spatial Y-maze, it was found that they were impaired if the interval between the trials was short (1 min) (Sanderson et al., 2009). In comparison, GluR1 knockout mice presented with enhanced spatial memory if the

interval between the trials was long (24 h), and this enhancement was caused by the interval between the exposure trials as opposed to prior to the test, which is suggestive of a genuine enhancement of learning and not simply enhanced performance (Sanderson et al., 2009). The fact that GluR1 deletion was able to both impair short-term spatial memory and enhance long-term memory, suggests that these forms of memory depend on separate psychological processes that can, under some circumstances, compete with one another. The behaviour of the Tc1 mice provide further support of the dual-process model of memory, and in addition support the idea of a role of GluR1 receptors as mediators of short term memory processing.

Wagner's SOP (1981) can again accommodate this result. Sanderson et al., (2009) suggest that GluR1 deletion may impair short-term learning by affecting the rate of transfer between the A1 and the A2 state. For example, the deletion of GluR1 may result in a reduction in the rate at which elements are transferred from A1 to A2, or reduce the capacity of A2, or alter the extent to which elements remain active in the A2 state, all of which would impair short-term memory due to a reduction of stimulus elements in the A2 state. Of course, in addition to this, there would then be an increased number of stimulus elements available in the A1 state, allowing for further opportunity for the formation of long-term associations. However, unlike the study by Sanderson et al., (2009) there is no enhancement of long term recognition memory in the Tc1 mice as there is in the GluR1 knockout mice. Perhaps the reduction in GluR1 receptor expression is sufficient to cause a deficit in short-term memory processes, but the lack of complete knockout of GluR1 results in the lack of an enhancement of long-term memory processes. A potential hypothesis as to why GluR1 is reduced in the Tc1 mouse model will be reserved for the general discussion.

With regards to kainate receptor subunit expression, the data revealed no significant alterations in GluK1 in the hippocampus or perirhinal cortex. The GluK1-containing kainate receptors are important for regulating synaptic facilitation and LTP induction at hippocampal mossy fibre synapses (Nistico et al., 2009). GluK1-containing glutamate receptors form Ca2+ channels that are expressed in the hippocampus, cerebellum and cortex, and the GluK1 gene maps to chromosome 21 (Ghosh, Sinha, Chatterjee & Nandagopal, 2009). In relation to individuals with DS, there is evidence for polymorphisms in GluK1 kainate receptors (Ghosh, Sinha, Chatterjee, & Nandagopal, 2009). There is also evidence for decreased microtubule motor protein KIF17 expression in trisomic mice, which may alter the distribution of GluK1 localization in distal dendrites (Kayadjanian, Lee, Pina-Crespo, & Heinemann, 2007; Roberson, Toso, Abebe, & Spong, 2008). Thus, although there is no evidence of reduced GluK1 expression, this may not reflect the activity of the receptors; it may be that the GluK1 distribution is

altered, or that the functionality of the GluK1-containing kainate receptors will be altered in Tc1 mice, and further experiments would need to be carried out to investigate this.

Expression of GluK5 subunit expression on the other hand, was found to be reduced in the perirhinal cortex. The reason for this reduction in expression is not immediately clear, and further work will need to be done to ascertain the upstream processes of GluK5 expression which may be affected by trisomy-21. However, regardless of the, the impact of the reduction is particularly interesting. The potential cognitive role of kainate glutamate receptors previously remained unclear because of a lack of selective antagonists. However, the development of a selective GluK5 subunit kainate receptor antagonist UBP302, has allowed for the role of these receptors to be tested (More et al., 2004), and may implicate GluK5 receptors in the interesting pattern of behaviour which is displayed by Tc1 mice. Similar dissociations between short- and long-term recognition memory to those observed in Tc1 mice have been reported following manipulation of kainate receptors in the perirhinal cortex. Barker et al. (2006) reported that infusion of a kainate receptor antagonist UBP302 (a selective GluK5 antagonist) into the perirhinal cortex of rats impaired recognition memory following a short (20-min) delay but not following a long (24-h) delay. Interestingly, the observed impairments were in the acquisition of information because memory was not impaired when UBP302 was infused after acquisition. This suggests that the acquisition of short term memory for object information is kainate receptor (specifically GluK5) dependent.

The specificity in the reduction of GluK5 localised to the perirhinal cortex in the Tc1 mice, together with the impairment of Tc1 mice on short term novel object recognition memory tasks provides further weight to the argument that there is aberrant perirhinal cortex activity in the Tc1 mouse model. There is of course, the possibility that long term recognition memory is being supported by another brain region, such as the hippocampus. However, Barker et al., (2006) considered that any UBP302-induced impairment at the long delay was masked by memory processes that was independent of the infused region. This seemed implausible because when the perirhinal region was infused with AP-5, a selective NMDA antagonist, there was a memory impairment at the long delay. Thus, operation of the infused region; likely the perirhinal cortex was essential to recognition memory, and no other region appeared to compensate. These data also suggest that there is more than one recognition memory mechanism operating within the perirhinal cortex. Recognition memory at long term delays is intact in both the Tc1 mice, and in mice who have had a selective GluK5 antagonist infused into the perirhinal cortex. Thus, the reduction in GluK5 kainate receptor subunit expression may be a factor affecting the short term recognition memory performance of Tc1 mice.

Drug 9a has previously been shown to have cognitive enhancing effects in rats performing the novel object recognition task (unpublished data), and the data presented in this chapter provide increased evidence towards a positive cognitive enhancing effect of drug 9a. It will be crucial to gain more insight into the activity of AMPA receptors in the Tc1 mouse model during object novelty recognition with and without drug administration. In addition, it would also be of interest to investigate the activity of critical brain regions via *c-fos* activation, in both the absence and the presence of the drug, in order to truly ascertain whether any possible increase in AMPA receptor activity is translated to an increase in brain region activity in a way which may mirror the behavioural changes which are evident.

Critical to note is the lack of improvement in the WT mice with administration of drug 9a. Unlike the preliminary rat experiments, whereby typically functioning rats displayed improvement on the novel object recognition task when drug 9a was administered (unpublished data), there was no improvement in WT control mice as reflected by discrimination ratio scores. This lack of improvement however this may be due to the presence of a ceiling effect on this novel object recognition task. Perhaps if task difficulty were to be increased, so that WT performance were not already at ceiling, then there may be a detectable improvement in WT control animals. The presence of this proposed ceiling effect was supported by the lack of improvement in WT animals under administration of Modafinil. Although Modafinil failed to improve performance of the mice in the novel object recognition effect, further suggesting that the ceiling effect could be real, further studies could use a 128mg/kg dose of Modafinil. In addition, Modafinil failed to cause any improvement in the performance of Tc1 animals on the novel object recognition task, which provides important information about the specificity of drug 9a. Modafinil has been shown to work via a number of systems; histamine, serotonin, GABA (Ishizuka et al., 2003; Ferraro et al., 2013; Ferraro et al., 1996; Ferraro et al., 1997). This experiment may begin to start ruling out some of these systems as not underlying the changes seen in the Tc1 mice.

Crucially, the effect of drug 9a does not increase exploration time of Tc1 mice in the sample phases; although Tc1 mice are more exploratory than WT animals, this is not an effect which is further increased with administration of the AMPAkine. This suggests that the positive effect of drug 9a it is not merely due to increased exploration of objects, but is instead likely to be enhancing the efficiency of learning and/or memory. In contrast, the exploration time of animals administered with Modafinil is increased, but nevertheless thus did not facilitate performance during the novelty test.

The pattern of findings relating to GluR1, GluK1 and GluK5 subunit expression are consistent with the hypothesis that AMPA and kainate receptor mechanisms are important mediators of synaptic plasticity, particularly concerning short term recognition memory processes. The reduction of the GluK5 receptor subunit expression in the perirhinal cortex, and the trend toward a reduction in GluR1 expression in the perirhinal cortex of Tc1 mice provide a potential synaptic mechanism for the deficit in Tc1 short term recognition memory. Additionally, the short term recognition memory deficit which is present in Tc1 mice, is no longer apparent in the presence of drug 9a, a novel positive allosteric modulator of the AMPA receptors. Further studies investigating the activation of AMPA and kainate receptors would help to elucidate the role of AMPA and kainate receptor subunits in different types of learning and memory.

6. General Discussion

Thesis Overview

The main aim of this thesis was to investigate recognition memory and processing in the MTL of the Tc1 mouse model of trisomy-21. The goal was to provide insight into the learning and memory changes associated with triplication of genes on human chromosome 21 in mice. This thesis also aimed to explore some of the biological systems underpinning the pattern of learning and memory changes demonstrated by the Tc1 mouse, specifically the perirhinal cortex and the hippocampus. Finally, this thesis investigated the expression levels of the GluR1 subunit of the AMPA receptor, as well as the GluK1 and GluK5 subunits of the kainate receptor in Tc1 mice, before finally investigating the impact of the administration of a novel AMPAkine, drug 9a, on the behavioural profile of the Tc1 mouse on the novel object recognition task. This chapter will summarise the main findings, followed by a more detailed discussion of the theoretical implications of the results and how these findings impact our understanding of learning and memory processes in trisomy of human chromosome 21.

Summary of Main Findings

Previous work has demonstrated that the Tc1 mouse displays intact long term recognition memory, but a deficit in short term recognition memory (O'Doherty et al., 2005; Morice et al., 2008). The aim of the first experiment, therefore, was to test the hypothesis that the Tc1 mouse model would demonstrate intact long term recognition memory, and a deficit in short term recognition memory. First, we examined short and long-term recognition memory in Tc1 mice, and in addition, extended the investigation to include an "immediate" (1 min) delay period. The data revealed that Tc1 mice do indeed show a deficit on short term (10 min) object recognition memory, but intact long term (24 hour) recognition memory and intact immediate (1 min) recognition memory. Thus, Tc1 mice showed an interesting U-shaped pattern of recognition memory performance. The recognition memory task was also extended to include olfactory information and demonstrated that Tc1 mice have impaired short term but intact long term olfactory recognition memory. The specificity of the behavioural deficits in Tc1 mice was confirmed using APP overexpressing mice, the Tg2576 mouse model, which showed the opposite pattern of object memory deficits with intact short term, and deficits in long term object recognition memory. Chapter 3 also examined memory for spatial information to test the hypothesis that aberrant hippocampal function in Tc1 mice would disrupt memory for the spatial organisation of objects. In contrast to object memory, Tc1 mice showed no deficit in either immediate or long-term memory for object-in-place information. Similarly, Tc1 mice showed no deficit in short-term memory for object-location information. The latter result indicates that Tc1 mice were able to detect and react to spatial novelty at the same delay interval that was sensitive to an object novelty recognition impairment. Finally, the data from chapter 3 also revealed intact performance of the Tc1 mice on a temporal order recognition memory task.

Chapter 4 examined whether Tc1 mice displayed a deficit in short term novel object recognition when retrieving object information from long term memory. The experiments in chapter 4 demonstrated that Tc1 mice have an overall impairment on the novel object recognition task ten minutes after presenting a retrieval cue using either familiar objects exposure to the training context 24-hours after the initial exposure. Although Tc1 mice performed above chance level, performance was still reduced in comparison to that of WT animals, and subsequent experiments indicated a more robust deficit on the NOR task in Tc1 mice following a ten minute delay. Chapter 4 also investigated patterns of neuronal activation in the perirhinal cortex and hippocampus following a memory retrieval cue, using the immediate early gene, *c-fos*. The results demonstrated potentially altered

perirhinal cortex activity, and in the CA3 region of the hippocampus of Tc1 mice, though further work will be required to confirm this.

Chapter 5 aimed to investigate the expression levels of the GluR1 AMPA receptor subunit, and the expression levels of GluK1 and GluK5 kainate receptor subunits in the hippocampus and perirhinal cortex of Tc1 mice. Results revealed no alteration in GluK1 expression in either the hippocampus of the perirhinal cortex. The GluK5 subunit however, although showing no change in expression pattern in the hippocampus, showed a reduction in the perirhinal cortex of Tc1 mice when compared to control mice. The GluR1 AMPA receptor subunit showed a reduction in expression in the hippocampus, and although not statistically significant, there was a trend. Finally, the administration of a novel AMPAkine, drug 9a, eradicated the significant difference in performance between Tc1 and WT control mice on the novel object recognition task following a 10 minute delay, unlike administration of a vehicle dose, or administration of Modafinil.

Discussion of Main Findings

The role of the perirhinal cortex in recognition memory

There is a great deal of work which highlights a role for the perirhinal cortex in recognition memory; however, there is still controversy about the magnitude of the role played by the hippocampus (Brown and Aggleton, 2001). Although many studies have provided evidence which dissociates the roles of the perirhinal cortex and hippocampus, these two regions also have functional interactions (Barker and Warburton, 2011). Disconnection of the hippocampus from either the perirhinal or medial prefrontal cortex caused impairments in object-in-place and in temporal order tasks, but had no effect on the object location or object recognition tasks. Thus, it appears that judgement of familiarity of objects themselves lies with the perirhinal cortex. In contrast, recognition memory involving the contextual associations of items, or the temporal order in which they are encountered, depends on interactions between the perirhinal cortex, hippocampus, and medial prefrontal cortex. Thus, there is a recognition memory network, in which the hippocampus plays a role when memories involve information that has a spatial component, or a recency element.

Given the dissociation between the types of recognition tasks on which the Tc1 mice demonstrate a deficit, we can make some assumptions about the network problems encountered by the Tc1 mice. The Tc1 mice show a delay dependent deficit in object recognition memory, but intact performance on object location, object-in-place and temporal order tasks. Taking evidence from Barker and Warburton (2011) into account, Tc1 mice show no deficits on object location or object in place tasks; tasks that have been demonstrated to be hippocampal dependent, or which rely on a functional connection between the hippocampus and perirhinal cortex, suggesting that this network is intact. The pattern of behaviour demonstrated by the Tc1 mouse suggests that the perirhinal cortex alone, is not functioning normally with regards to object information. The delay dependent deficit also provides further strength to this argument, as hippocampal lesions have been shown to affect object recognition more consistently at long delays and not short delays. Thus, intact object recognition memory performance in the Tc1 mice at 24 hours may point toward intact hippocampal function which is compensating for aberrant perirhinal cortex function at the long term delay.

The involvement of the GluR1 AMPA receptor subunit in recognition memory

In attempting to understand the biological systems underpinning the pattern of recognition memory deficit in the Tc1 mouse model, we investigated the expression profile of the GluR1 receptor, which has been repeatedly implicated in learning and memory in mouse models. The dissociable properties of short and long term memory in the Tc1 mouse provide support for dissociable memory processes, as opposed to a model whereby short term memories are serially converted into long term memories. Similarly, GluR1 knockout mice showed impaired performance on tests of spatial recognition memory when the interval between trials was short (1 min), but showed enhanced spatial memory if the interval between the trials was long (24 h), which also supports the idea of separate short and long term memory processes. Findings by Morice et al., (2008) had already demonstrated a reduction in surface receptor expression of the GluR1 subunit in the DG of the hippocampus in Tc1 mice, however, considering that DG lesions have been shown to affect spatial recognition memory (Gilbert, Kesner & Lee, 2001), and the deficit seen in Chapter 3 was on tasks of object recognition, with intact recognition of spatial elements, it was also appropriate to explore the expression patterns of GluR1 in the perirhinal cortex of Tc1 mice.

The pattern of GluR1 expression in the Tc1 mouse model was found to be reduced in all regions of the hippocampus (CA1, CA3 and DG). Thus, the reduction of GluR1 expression in the DG was replicated. In addition, although the reduction in GluR1 expression in the perirhinal cortex was not significant, there was a trend towards a reduction in this brain region. This trend could be further explored using a more sensitive technique, such as an enzyme-linked immunosorbent assay (ELISA) in order to confirm whether or not this trend is reflective of a real change in GluR1 receptor expression in the perirhinal cortex.

In the same way as the Tc1 mice, GluR1 knockout mice have been shown to display impaired performance on the object recognition task (Sanderson et al., 2011). The novel object recognition deficits seen in GluR1 knockout mice were at short-term delays, and although Tc1 mice do not appear to have any significant spatial memory deficits, spatial memory deficits in GluR1 knockout mice show the same temporally dependent pattern of recognition memory deficits as that seen in Tc1 mice; intact long term with deficits in short term recognition memory. Thus, there are commonalities in the behavioural patterns shown between Tc1 and GluR1 knockout mice, and the fact that GluR1 expression levels were altered in Tc1 mice, albeit subtly in the perirhinal cortex, provided

justification for an attempt at AMPA receptor modulation in these animals as a way of improving object recognition memory performance.

With regards to an explanation as to why GluR1 expression may be reduced in Tc1 mice, it is possible that trisomy of the genes on chromosome-21 may cause some dysregulation of the processes upstream of GluR1 expression. Sorting nexin 27 (SNX27), is a brain-enriched protein which regulates endocytic sorting and trafficking, regulating their recycling to the plasma membrane. It has previously been demonstrated that SNX27 knockout mice showed severe neuronal deficits in the hippocampus and cortex, defects in synaptic function and deficits in learning and memory, including deficits on the novel object recognition task (Wang et al., 2013). However, the temporal properties of this test are not made clear in the paper. SNX27 mice also showed a reduction in the amounts of ionotropic glutamate receptors, including AMPA receptors (Wang et al., 2013). In the study by Wang et al., (2013), the levels of SNX27 in brain samples from humans with Down syndrome and age/gender matched controls were examined, and it was found that both protein and mRNA levels of SNX27 were markedly decreased in the cortex. Consistent with this finding, levels of GluR1 were also reduced. These findings were mirrored in the Ts65Dn mouse model (Wang et al., 2013). Wang et al. (2014) went on to discover that the chromosome 21-encoded miR-155 targets and down-regulates C/EBPβ, which is a transcription factor for SNX27. Thus, the lower levels of C/EBPβ in Down syndrome lead to reduced SNX27 expression. In addition, upregulating SNX27 in the hippocampus of the Ts65Dn mouse model of Down's syndrome rescues synaptic and cognitive deficits. Thus, the reduction of GluR1 in the hippocampus and perirhinal cortex of Tc1 mice could be linked to a putative loss of SNX27, as a result of overexpression of microRNA-155, and it will be interesting to examine the expression profiles of SNX27 in the brains of Tc1 mice in the future.

Dissociations between spatial and non-spatial recognition memory

Although a lot of the data presented in this thesis point toward abberant perirhinal cortex function in the Tc1 mouse model, previous research has focussed more on the hippocampus. Pennington et al., (2003) actually proposed the hippocampal hypothesis of DS, suggesting that cognitive impairment in DS is disproportionately influenced by hippocampal dysfunction. One prediction stemming from this hypothesis is that people with DS and mouse models of DS, would exhibit a greater degree of impairment in the spatial versus non-spatial recognition memory, a view which has been supported by data from human DS studies, and from studies on other mouse models such as the Ts65Dn.

Tc1 mice have been shown to exhibit significantly reduced early LTP compared with that of WT littermate controls (Morice et al., 2008; Witton et al., 2015). Morice et al., (2008) also provided evidence for a reduction in the expression of the GluR1 subunit of the AMPA receptor in the DG of Tc1 mice, which may be linked to the reduction in early LTP. Previous work also demonstrated abnormal hippocampal spine morphology, and sub-region changes in the connectivity of the DG-CA3 network that contributes to disruption of place-cell activity (O'Doherty et al., 2005; Witton et al., 2015). This, together with difficulties with spatial memory in DS humans (Pennington, Moon, Edgin, Stedron, & Nadel, 2003), led to the prediction that memory for the spatial organisation of objects would be disrupted in Tc1 mice (Burke et al., 2011; Lenck-Santini, Rivard, Muller, & Poucet, 2005). This hypothesis was not supported. Thus, as well as a temporal dissociation between short and long term object recognition memory in the Tc1 mice, there is also a dissociation between memory for spatial and non-spatial aspects of recognition memory. The results from this thesis demonstrate (1) that novelty detection per se and (2) the encoding of visuo-spatial information was not disrupted in adult Tc1 mice.

The pattern of behavioural deficits in Tc1 mice is clearly different from that shown by Ts65Dn mice (see table 6.1). The Ts65Dn mouse model is a segmental trisomy model of DS and mice are trisomic for approximately 56% of genes on mouse chromosome 16 that are homologues for human chromosome 21 (Ruparelia, Pearn, & Mobley, 2013). In contrast to Tc1 mice, several studies have reported Ts65Dn deficits in long-term (24-h) object recognition memory (Braudeau et al., 2011; Colas et al., 2013; Contestabile et al., 2013; De la Torre et al., 2014; Kleschevnikov et al., 2012; Lockrow, Boger, Bimonte-Nelson, & Granholm, 2011; Smith et al., 2014; Stringer, Abeysekera, Dria, Roper, & Goodlett, 2015). More specifically, Smith et al. (2014) showed that short-term memory, but not long-term recognition memory, was intact when the Ts65Dn mice were tested in an environment with minimal extramaze cues. When extramaze cues were available, short-term object recognition memory was also impaired in Ts65Dn mice. Other studies have reported object-location memory deficits in Ts65Dn with delays between 10-min- and 24-h (Contestabile et al., 2013; Hyde & Crnic, 2002; Kleschevnikov et al., 2012).

The difference between the recognition memory pattern shown by the Ts65Dn and the Tc1 mouse is perhaps not surprising when the genetic differences between the two models are taken into account. The models differ in a number of respects; Tc1 mice express approximately 75% of the genes of Hsa21 (Choong, Tosh, Pulford, & Fisher, 2015), however they also possess a deletion, 6 duplications and more than 25 de novo structural rearrangements of Hsa21 (Gribble et al., 2013). Ts65Dn possess three copies

of the segment of chromosome 16 that is orthologous to a critical region of Hsa21 (Davisson, Schmidt, & Akeson, 1990) and 79 other genes on chromosome 17 that are outside the syntenic region of Hsa21 (Choong et al., 2015).

One other important difference between the two models is that the amyloid precursor protein (APP) is not trisomic in Tc1 mice, whereas it is present in three copies in Ts65Dn mice (Choong et al., 2015). APP plays a major role in brain development and neurogenesis and APP trisomy may contribute to abnormal brain development and cognition (Cataldo et al., 2003; Giacomini et al., 2015; Trazzi et al., 2014). In this context, it is interesting to note that Tg2576 mice showed the opposite pattern of memory deficits to Tc1 mice, with a deficit in long term recognition memory, and intact short term recognition memory; a pattern that was arguably more similar to that shown by Ts65Dn mice (Good & Hale, 2007). Although we compared mice expressing a mutant form of APP, the pattern of results suggests that aberrant APP expression may contribute to impaired long-term object and place recognition memory deficits in Ts65Dn and Tg2576, and that other genes may be responsible for the impact on short-term (recognition) memory processes in the Tc1. It would also appear that trisomy for different regions of chromosome 21 to those that are trisomic in the Tc1 mouse, or perhaps the interaction of specific mutations with an overexpression of APP may have a negative influence on spatial recognition memory in ways that are not evident in the Tc1 mouse. Further behavioural assessment of mouse models trisomic for different regions orthologous to Hsa21 will help to address this question.

Of course, although there is no overwhelming evidence for a spatial deficit in the Tc1 mouse model presented in this thesis, there is of course the possibility that the tests were not sensitive to a subtle spatial memory impairment. For example, GluR1 knockout mice specifically showed a deficit on spatial working memory tasks, as opposed to reference memory tasks (Sanderson et al., 2009). Although the object location and object-in-place tasks used in this thesis may indeed involve working memory, in the sense that information is acquired over 1-2 trials, the mice only perform the task once, therefore, unlike some tests of working memory there is no proactive interference to hinder working memory. Similarly, Tc1 mice have been reported to show normal learning in a standard version of the water maze and normal spontaneous alternation in a T-maze, a rule- and reinforcement-free test of innate behaviour (Morice et al., 2008). Perhaps if a more complex working memory task were used, which requires disambiguation of multiple rewarded locations, changes in spatial working memory in the Tc1 mouse may become evident. Indeed, in a study by Witton et al., (2015) Tc1 mice were tested on the six-arm radial arm maze task, and were slower than wild-type littermates to attain an efficient level of spatial working memory performance. Notably, the performance of Tc1 animals

was significantly different to WT animals on only one trial block. As Witton et al., (2015) point out, further testing will be required to determine whether Tc1 behavioural impairments reflect load-dependent visuospatial working memory deficits.

A comparison of Tc1 and the Ts65Dn mice as models of Down syndrome		
	Tc1	Ts65Dn
Genetics	Contains an almost entire copy of human chromosome-21	Trisomic for the distal end of Mmu 16 and a small portion of Mmu 17
LTP	Reduced early LTP but normal late LTP in the DG. Intact in CA1 and CA3.	Reduced in the CA1 and DG. Intact in the CA3.
Receptor Changes	Reduced GluR1 in hippocampus Reduced GluK5 in perirhinal cortex	Increased GABA inhibition in hippocampus Increased phosphorylation of GluR1 in hippocampus
Novel Object Recognition	Impaired short term, intact long term	Intact short term, impaired long term
Object in Place	Normal (immediate and 24 hour)	Normal
Object Location	Normal (10 minutes)	
Spatial working memory (Radial Arm Maze)	Impaired	Impaired
Temporal Order	Normal	
Morris Water Maze (Cued)	Normal	Impaired
Morris Water Maze (Hidden Platform)	Normal	Impaired
Contextual Fear Conditioning		Impaired

Table 6.1 *– Table of the key phenotypic differences between the Tc1 and Ts65Dn mouse models.*

Dissociation between short- and long-term object recognition memory processes

One of the most interesting aspects of the pattern of behaviour shown by the Tc1 is the very specific temporal nature of the recognition memory deficit; intact immediate and long term object recognition memory, despite a deficit in short term object recognition memory following a ten minute delay. We have speculated that disruption to GluR1 receptors may contribute to this alteration in memory processes. However, other receptor or neurotransmitter changes may also underlie this temporal pattern of memory in mice.

Tc1 mice show intact performance on spatial recognition tasks, and object recognition tasks at long term delays. Given evidence that antagonism of NMDA receptors in rodents has been shown to impair novel object recognition tasks at delays of more than 1 hour (Barker et al., 2006), this suggests that NMDA receptor activity in the Tc1 mouse is normal. Kainite and muscarinic receptor antagonism in the perirhinal cortex on the other hand, has been shown to impair novel object following delays of less than 1 hour (Barker et al., 2006; Tinsley et al., 2011). Thus, it could be argued that that kainate or muscarinic receptors are not functioning as they should in the Tc1 mouse. The results presented in this thesis, did not reveal any alteration in GluK1 expression, however, there was a significant reduction of GluK5 expression in the perirhinal cortex. GluK5 alterations could alter the subunit composition and properties of heteromeric ion channels and have a detrimental effect on the short-term recognition memory in Tc1 mice. Barker et al. (2006) had interestingly reported that infusion of a selective GluK5 kainate receptor subunit antagonist into the perirhinal cortex of rats impaired recognition memory following a short (20-min) delay but not following a long (24-h) delay; a pattern akin to that observed in the Tc1 mouse.

Taken together, the findings from the present thesis, and from Barker et al., (2006), suggest that there is more than one recognition memory mechanism operating within the perirhinal cortex. That is, as recognition memory at long term delays is intact in both the Tc1 mice and in mice who have had a selective GluK5 antagonist infused into the perirhinal cortex. Thus, the reduction in GluK5 Kainate receptor subunit expression may be a factor affecting the short term recognition memory performance of Tc1 mice. Collectively these data indicate that object novelty memory is sensitive to different receptor mechanisms in a delay-dependent manner. There is currently no data regarding muscarinic receptor expression or activation in the Tc1 mouse, and further work will be required to ascertain whether cholinergic innervation is intact in the Tc1 mouse.

Habituation and Recognition Memory

Throughout this thesis, much of the data has been discussed within the model of Wagner's SOP (1981). Specifically, that in Tc1 mice there are a higher proportion of familiar stimulus elements available in the A1 state and this contributes to the greater exploration of these objects. This may result from a slower decay of stimulus elements from the A1 to the A2 state, or a more rapid decay of elements from the A2 state into the inactive state where they become available for activation into the A1 state. However, a major prediction of this model and its application to Tc1 mice is that there should be a deficit in habituation. That is, upon the subsequent presentation of objects in sample phases, there would still be a higher proportion of familiar stimulus elements available in the A1 state, and thus, exploration of the objects in sample phases, both within and across days would remain higher in Tc1 mice.

However, this was not the case. Consistently, Tc1 mice showed evidence of habituation to the objects across sample phases. This presents a dilemma when interpreting the short term object recognition deficit, as although Tc1 mice consistently present with a deficit in object recognition following a short (10 minute) delay, the problem cannot be a simple short term memory problem if they show intact habituation across sample phases following a short (10 minute) delay.

A key difference between the deficit shown in object recognition tests, and habituation is the process of discrimination. During the second sample phase, whereby habituation is evident, the mice are presented with the same three items as they were presented with in sample phase one; all presented objects are familiar. Crucially, in the test phase, mice are presented with two familiar objects and one novel object; they are required to make a "judgement" about whether and object is familiar or novel.

Olarte-Sanchez et al., (2015) were also puzzled by the fact that rodents often show intact habituation despite a subsequent recognition deficits. They suggested that the perirhinal cortex may be driving the discrimination between novelty and familiarity, and that perirhinal lesions disrupt the ability to distinguish the particular source of novelty signals. Furthermore, they suggested that there may be another system which ensures that information that a novel object is present remains intact, thereby preserving exploration levels. To test this hypothesis, the performance of rats with perirhinal cortex lesions was contrasted in two behavioural procedures; the first tested the spontaneous preference for novel objects over familiar objects, when both are presented simultaneously, whilst the second involved sequentially presenting pairs of objects that were either both novel

or both familiar. The measure of intact recognition memory in experiment two assumed that there would be higher exploration levels for a pair of novel objects over a pair of familiar objects. The study revealed that, as would be anticipated, perirhinal cortex lesions resulted in poor discrimination between novel and familiar stimuli when presented together. In contrast, the same rats with perirhinal cortex lesions performed normally when tested sequentially, with either only novel, or only familiar items. This suggests that in the absence of the perirhinal cortex, rats still receive signals of object novelty, which they may fail to assign to the appropriate object, possibly resulting in the treating of familiar objects as novel, and leading to deficits on tests of recognition when a discrimination judgement must be made.

Olarte-Sanchez et al., (2015) also reported that there was no indication that perirhinal cortex lesions reduced total levels of object exploration for novel objects, which would be the case were the lesions causing novel stimuli to appear familiar. This is interesting regarding the Tc1 animals, as the contact time data demonstrates that they do not treat the novel objects as familiar, but rather, the familiar objects as novel. Thus, the aberrant perirhinal function in Tc1 mice, together with normal habituation, provides further support for Olarte-Sanchez et al., (2015). If one were to assume one system for detecting familiarity, and another for discriminating between novel and familiar objects, this would explain why Tc1 mice are capable of habituating to familiar items, but are incapable of discriminating between novelty and familiarity. The aberrant perirhinal cortex activity seen in Tc1 mice, paired with poor discrimination supports the suggestion that the perirhinal cortex is the structure driving the discrimination between novelty and familiarity.

Further to this argument, it is interesting to note that data from chapter 3 demonstrate that Tc1 mice showed no deficit on the temporal order recognition memory task. This lends support not only to the idea that long term memory is intact in the Tc1, but also that they are able to make some judgements about items seen simultaneously; they are capable of discriminating between two items which have been seen at different times, thus they can make an old vs. new discrimination. It would appear then that the discrimination problem in the Tc1 mice is specific to a novel vs. familiar judgement, which Olarte-Sanchez et al., (2015) suggest is a function specifically related to the perirhinal cortex.

It would be interesting to investigate the performance of the Tc1 mice on a serial novel object recognition task, whereby they would be presented with a pair of novel objects, followed by a pair of the same (now familiar) objects, followed by a pair of novel objects,

and then a pair of objects in which one object was novel and one familiar, and so on. The hypothesis would be that Tc1 mice would show a reduction in exploration when faced with two familiar objects, but would have difficulty discriminating between the novel and familiar objects when presented simultaneously, as this would require a judgement between novelty and familiarity, as opposed to simply familiarity detection. The Albasser et al., (2010) bow-tie maze would provide a useful protocol for this, as it allows multiple trials within a session. This experiment would provide further insight into the learning and memory abilities of the Tc1 mice, and would further establish the whether there is indeed aberrant perirhinal cortex function in the Tc1 mouse model.

Drug 9a as a therapeutic tool in DS

This thesis revealed commonalities in the behavioural deficits demonstrated by both the Tc1 and the GluR1 knockout mice, together with a significant reduction in GluR1 receptor expression in the hippocampus, and a trend toward a reduction in the perirhinal cortex. Based on these similarities, the hypothesis that facilitation of AMPA receptor mediated synaptic activity would be beneficial to recognition performance in the Tc1 mice.

The administration of drug 9a, a positive allosteric modulator of the AMPA receptors, had positive effects on the short term recognition memory in the Tc1 mice. This is suggestive of a positive cognitive enhancing effect of drug 9a on Tc1 mice. With regards to why this effect takes place, and how exactly the AMPAkine is acting, it will be crucial to gain further understanding of the cellular correlates of this behavioural change. A series of experiments which uses in vivo ensemble recording, with electrodes placed in the hippocampus and perirhinal cortex to monitor activity during object recognition tasks, both with and without drug 9a on board would help to elucidate this.

Previously developed AMPAkines have been shown to have positive physiological and behavioural effects. AMPAkines produce large increases in neuronal activity in discrete cortical regions and networks engaged by particular behavioural demands (Hess et al., 2003), and they have also been shown to increase the degree and duration of long-term potentiation (Staubli, Rogers & Lynch, 1994). Studies have also demonstrated that AMPAkines elevate production of brain derived neurotrophic factor (Lauterborn et al., 2000). At the level of memory and cognition, short-term (minutes) memory scores were increased in rats performing a delayed non-match to sample problem (Hampson, Rogers, Lynch & Deadwyler, 1998), and AMPAkines have also been shown to accelerate the encoding of long-term (days) memory; AMPAkines reduced the number of trials needed by rodents to acquire an odour discrimination (Larson et al., 1995). In addition,

AMPAkines approximately doubled the rate at which rats acquired amygdala dependent fear learning (Rogan, Staubli & LeDoux, 1997). With regards to animal models of psychiatric disorders, AMPAkines have been reported to reduce the hyperactivity phenotype in a mouse model of attention deficit hyperactivity disorder (Gainetdinov, Mohn, Bohn & Caron, 2001), and anti-depressant effects in mouse models of depression (Li et al., 2001). Their use is varied, however, a lot of previous attempts at modulating AMPA receptor activity has proved difficult; molecules which are able to act directly on AMPA receptors have been found to be poorly tolerated as they lead to uncontrolled central stimulation, causing seizure activity. Drug 9a however, has been shown to be potently efficacious with an attractive safety profile in preclinical species (Ward et al., 2011). Given the apparent beneficial effects of drug 9a in the Tc1 mouse, there is every potential to believe that the positive allosteric modulator may have positive cognitive enhancing effects in other mouse models. There may also be other positive effects of the drug on other tasks which are unmeasured in this thesis. The effects of drug 9a in other models of Down syndrome should be tested in order to investigate whether it would have positive effects on other types of recognition memory, such as the spatial recognition deficits seen in the Ts65Dn model, which are more closely aligned with what is seen in the human condition. Following further assessment of the drug in rodent models, the drug would be an interesting candidate for clinical trial, with potential to improve the cognitive abilities of DS patients.

How useful is the Tc1 mouse model?

Despite the presentation of some psychologically interesting results regarding dissociations in properties of recognition memory, the Tc1 mouse model of course has its limitations. Tc1 mice express approximately 75% of the genes of Hsa21 (Choong, Tosh, Pulford, & Fisher, 2015), however they also possess a deletion, 6 duplications and more than 25 de novo structural rearrangements of Hsa21 (Gribble et al., 2013). Therefore, the Tc1 mouse model is not a 100% recapitulation of the human condition. It may well be that the culmination of genes which are preserved in the Tc1 mouse model, in combination with the absence of genes which would be present in a full trisomy of chromosome 21, may well have created a unique syndrome which is not truly reflective of DS. As a result, in collaboration with the LonDownS consortium, a great deal of effort has been assigned to closely aligning the mouse and human tests of memory in order to get as accurate a picture as possible about the usefulness of this mouse model. Preliminary data from an object recognition study in DS infants, following an eye-tracking version of the protocol described in this thesis, has produced the same pattern of results as demonstrated in the Tc1 mouse model; intact long term, but a deficit in short term

recognition memory (personal communication, Annette Karmiloff-Smith, 2015). However, further behavioural assessment of mouse models trisomic for different regions orthologous to Hsa21 will help to narrow down the specific genes which are causing specific phenotypes when they appear in trisomy.

Thesis Summary and Conclusions

This thesis provides novel and valuable data in its demonstration that a mouse model of human trisomy 21 shows a selective deficit in short-term recognition memory while sparing long-term memory for the same type of information, and that this pattern generalises across stimulus modalities. This thesis also demonstrates for the first time that the expression of a near complete copy of human chromosome 21 in Tc1 mice does not impair place recognition when using object-in-place or object-location tasks. In addition, there is evidence for aberrant perirhinal cortex activity in response to familiarity, as measured by *c-fos* expression as a marker of neuronal activation. Further to this, there is evidence for a significant reduction of expression of the GluR1 AMPA receptor subunit in the hippocampus, and a trend towards a decrease in the perirhinal cortex, as well as a significant reduction of the GluK5 kainate receptor subunit expression in the perirhinal cortex. Finally, this thesis provides some preliminary evidence that a novel positive allosteric modulator, drug 9a, has a positive cognitive enhancing effect. Drug 9a eradicated the significant difference in performance between the Tc1 and WT mice on a novel object recognition task at a ten minute delay.

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