The development of spatial memory processes and associated cellular correlates in juvenile rats

By

Nikolaos Tzakis

A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Neuroscience

Carleton University

Ottawa, Ontario

© 2019
Nikolaos Tzakis

Abstract

The late postnatal period is marked by neurodevelopmental changes, from gross morphological alterations to finite cellular and molecular variations. The hippocampus is one such structure that undergoes extensive morphological and cytological remodeling during the juvenile period, which is thought to mediate the maturation of cognitive processing.

Hippocampal remodeling leads to the strengthening of intra-hippocampal and hippocampal-cortical connectivity, allowing for the consolidation of information into remote stores within extrahippocampal regions. A link between changes in the duration of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA)-mediated synaptic responses in the hippocampus and the emergence of spatial navigation has been shown. AMPA are made up of 4 subunits, of which GluR1 and GluR2 have been shown to play the most prominent role in cognitive processes. The current thesis investigated whether preadolescent spatial memories acquired during this period of hippocampal synaptic modification persist beyond 24 hours and stabilize into the postadolescent period. We further aimed to identify the neural correlates thought to underlie the emergence of such processes, while determining training factors that may facilitate spatial memory processing. To this extent, rats were trained on spatial memory tasks during the period of hippocampal remodeling and assessed at either recent (24h) or remote (3 week) testing intervals. Their performance on the tasks was compared to adults in order to delineate the developmental period in which memory processing in juveniles resembles that in adults. c-Fos immunohistochemistry was performed on both the hippocampus and anterior cingulate cortex (ACC) in order to determine their contributions to testing performance at either interval, while Western blotting in both regions identified GluR1 and GluR2 levels following testing. Results indicated that the emergence of recent memory processing coincided with the

initial onset of hippocampal remodeling, while remote memory processes showed a protracted development, manifesting during late-phase hippocampal remodeling. The pattern of memory consolidation suggests that the hippocampus is initially responsible for the processing of recently acquired information, after which it is stabilized and consolidated into remote stores within the ACC, rendering it independent of hippocampal activity. Both recent and remote memory processing are likely mediated by the development of synaptic components, particularly GluR1 and GluR2.

Co-Authors

Matthew R. Holahan

Acknowledgements

I would like to express my deepest gratitude to my incredible supervisor, Dr. Matthew Holahan, for his continued support, commitment, guidance, and advice throughout my graduate school career. I would also like to thank my committee, Dr. Natalina Salmaso and Dr. Hongyu Sun for their guidance. To Brendan Hoffe, Tim Bosnic, Thomas Ritchie, and Tanisse Teale, thank you for your support with each of the experiments in this thesis. To my lab mates, thank you for being there for encouragement and support whenever it was needed.

Dedication

I would like to dedicate this thesis to all my friends and family who have been there to support me the whole way. To Stephen, whose constant love, emotional support, and patience has helped me persevere to the end. To Robyn, whose always been there for me. And above all, to my parents, Aristotle and Line, whose incredible, undying support throughout my entire student career has helped me achieve one of the greatest goals of my life.

Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ACC	Anterior cingulate cortex
cAMP	Cyclic adenosine monophosphate
CRE	cAMP response element
CREB	cAMP response element-binding protein
CA	Cornu Ammonis
CAMKII	Calcium/calmodulin-dependent protein kinase II
DCX	Doublecortin
DG	Dentate Gyrus
E	Embryonic day
EPSC	Excitatory postsynaptic currents
GABA	γ -aminobutyric acid
LER	Long-Evans rat
LTD	Long-term depression
LTP	Long-term potentiation
MAGUK	Membrane-associated guanylate kinases
mPFC	Medial prefrontal cortex
NSF	<i>N</i> -ethylmaleimide-sensitive fusion protein
NMDA	<i>N</i> -Methyl- <i>D</i> -aspartate
P	Postnatal day
PKA	cAMP-dependent protein kinase A
PKC	cAMP-dependent protein kinase C
PKM ζ	Protein kinase Mzeta
Ser831	Serine 831
Ser845	Serine 845
TARP	Transmembrane AMPA receptor regulatory proteins

Contents

Abstract	ii
Co-Authors	v
Acknowledgements	iv
Dedication	vi
Abbreviations	vii
List of Figures	xii

Chapter 1: Introduction

1. Overview	1
2. Hippocampal Anatomy	1
2.1. Functional divisions of the hippocampus	1
2.2. Anatomical subregions of the dorsal hippocampus	3
3. Hippocampal Development	6
3.1. Morphogenesis of the hippocampus	6
3.2. Cytogenesis of the hippocampus	9
3.3. Development of hippocampal circuitry	11
4. Spatial Memory	14
4.1. Defining spatial memory	14
4.2. Spatial memory & the brain	16
4.3. Assessing spatial memory	22
4.4. Spatial memory & the hippocampus	27
4.5. The hippocampus & the cognitive map theory	30
4.6. Cellular underpinnings of spatial memory	33
4.7. Hippocampal development& the emergence of spatial memory processes	41
4.8. The fate of newly acquired spatial memories	45
5. Remote Memory	53
5.1. Memory storage in the anterior cingulate cortex	53
5.2. Ontogeny of remote memory capabilities	57
6. Current Thesis	59
6.1. Objectives and hypotheses	59

Chapter 2: Factors That Influence the Processing of Juvenile-Acquired Spatial Memories in Rats During a Crucial Period of Hippocampal Development

Abstract	64
1. Introduction	66
2. Materials and Methods	68
2.1. Subjects	68
2.2. Apparatus	69
2.3. Water maze training	69
2.4. Water maze testing	70
2.5. Conditions	70
2.6. Immunohistochemistry	71
2.7. Quantification	72
2.8. Statistical analyses	73
3. Results	74
3.1. Juvenile vs. adult	74
3.1.1. Trial-by-trial latency	74
3.1.2. Trial-by-trial pathlength	77
3.1.3. Target quadrant	80
3.1.4. Target counter	83
3.1.5. Immunohistochemistry	86
3.2. Massed vs. spaced training	91
3.2.1. Trial-by-trial latency	91
3.2.2. Trial-by-trial pathlength	94
3.2.3. Target quadrant	97
3.2.4. Target counter	100
3.2.5. Immunohistochemistry	103
3.3. P20-22:remote vs. P22-24:remote vs. P24-26:remote	108
3.3.1. Trial-by-trial latency	108
3.3.2. Trial-by-trial pathlength	111
3.3.3. Target quadrant	114
3.3.4. Target counter	117
3.3.5. Immunohistochemistry	120
4. Discussion	125

5. Conclusion	132
---------------------	-----

Chapter 3: Investigation of GluR1 and GluR2 AMPA receptor subtype distribution in the hippocampus and anterior cingulate cortex of Long Evans rats during development

Abstract	140
1. Introduction	141
2. Methods	145
2.1. Subjects	145
2.2. Western blotting	145
2.3. Immunohistochemistry	148
2.4. Statistical analyses	148
3. Results	149
3.1. Western blotting	149
3.1.1. GluR1	149
3.1.2. GluR2	154
3.2. Immunohistochemistry	159
4. Discussion	169
5. Conclusion	174

Chapter 4: Assessing the development of juvenile remote memory processing using an open field task and c-Fos staining in the hippocampus and anterior cingulate cortex

Abstract	189
1. Introduction	190
2. Methods	193
2.1. Subjects	193
2.2. Open field	193
2.3. Immunohistochemistry	194
2.4. Western blotting	195

2.5. Statistical analyses	198
3. Results	199
3.1. Open field task	199
3.2. Immunohistochemistry	210
3.2.1. CA1	210
3.2.2. ACC	210
3.3. Western blotting	220
3.3.1. GluR1	220
3.3.2. GluR2	223
4. Discussion	226
5. Conclusion	235
Chapter 5: General Discussion	247
Conclusion	262
References	263
Supplemental Data	292

List of Figures

Chapter 1:

Figure 1.A.: Trial-by-trial latency (juvenile vs. adult)	75
Figure 1.B.: Trial-by-trial pathlength (juvenile vs. adult)	78
Figure 1.C.: Percent time spent in target quadrant (juvenile vs. adult)	81
Figure 1.D.: Percent time spent in target counter (juvenile vs. adult)	84
Figure 1.E.: Hippocampal c-Fos density (juvenile vs. adult)	87
Figure 1.F.: Representative HPC staining (juvenile vs. adult)	87
Figure 1.G.: ACC c-Fos density (juvenile vs. adult)	89
Figure 1.H.: Representative ACC staining (juvenile vs. adult)	89
Figure 2.A.: Trial-by-trial latency (massed vs. spaced)	92
Figure 2.B.: Trial-by-trial pathlength (massed vs. spaced)	95
Figure 2.C.: Percent time spent in target quadrant (massed vs. spaced)	98
Figure 2.D.: Percent time spent in target counter (massed vs. spaced)	101
Figure 2.E.: Hippocampal c-Fos density (massed vs. spaced)	104
Figure 2.F.: Representative HPC staining (massed vs. spaced)	104
Figure 2.G.: ACC c-Fos density (massed vs. spaced)	106
Figure 2.H.: Representative ACC staining (massed vs. spaced)	106
Figure 3.A.: Trial-by-trial latency (P20-22 vs. P22-24 vs. P24-26)	109
Figure 3.B.: Trial-by-trial pathlength (P20-22 vs. P22-24 vs. P24-26)	112
Figure 3.C.: Percent time spent in target quadrant (P20-22 vs. P22-24 vs. P24-26)	115
Figure 3.D.: Percent time spent in target counter (P20-22 vs. P22-24 vs. P24-26)	118
Figure 3.E.: Hippocampal c-Fos density (P20-22 vs. P22-24 vs. P24-26)	121
Figure 3.F.: Representative HPC staining (P20-22 vs. P22-24 vs. P24-26)	121
Figure 3.G.: ACC c-Fos density (P20-22 vs. P22-24 vs. P24-26)	123
Figure 3.H.: Representative ACC staining (P20-22 vs. P22-24 vs. P24-26)	123

Chapter 2:

Figure 1.A.: ACC GluR1 western blot analyses	150
Figure 1.B.: ACC GluR1 western blot representative bands	150
Figure 2.A.: HPC GluR1 western blot analyses	152
Figure 2.B.: HPC GluR1 western blot representative bands	152
Figure 3.A.: ACC GluR2 western blot analyses	155
Figure 3.B.: ACC GluR2 western blot representative bands	155
Figure 4.A.: HPC GluR2 western blot analyses	157
Figure 4.B.: HPC GluR2 western blot representative bands	157

Figure 5.A.: Representative HPC GluR1 staining	160
Figure 5.B.: Representative HPC GluR2 staining	161
Figure 6.A.: Dentate gyrus GluR1 immunohistochemical analyses	163
Figure 6.B.: Representative dentate gyrus GluR1 staining	164
Figure 7.A.: Immunohistochemical analyses of GluR2 in dentate gyrus	166
Figure 7.B.: Representative dentate gyrus GluR2 staining	167

Chapter 3:

Figure 1.A.: Total distance traveled during training and testing (P16)	203
Figure 1.B.: Total distance traveled during training and testing (P18)	203
Figure 1.C.: Total distance traveled during training and testing (P20)	203
Figure 1.D.: Total distance traveled during training and testing (P25)	203
Figure 1.E.: Total distance traveled during training and testing (P30)	203
Figure 1.F.: Total distance traveled during training and testing (P50)	203
Figure 2.: Difference score	205
Figure 3.A.: Ratio of distance traveled in center to total distance traveled (P16 - recent)	207
Figure 3.B.: Ratio of distance traveled in center to total distance traveled (P16 - remote)	207
Figure 3.C.: Ratio of distance traveled in center to total distance traveled (P18 - recent)	207
Figure 3.D.: Ratio of distance traveled in center to total distance traveled (P18 - remote)	207
Figure 3.E.: Ratio of distance traveled in center to total distance traveled (P20 - recent)	207
Figure 3.F.: Ratio of distance traveled in center to total distance traveled (P20 - remote)	207
Figure 3.G.: Ratio of distance traveled in center to total distance traveled (P25 - recent)	207
Figure 3.H.: Ratio of distance traveled in center to total distance traveled (P25 - remote)	207
Figure 3.I.: Ratio of distance traveled in center to total distance traveled (P30 - recent)	207
Figure 3.J.: Ratio of distance traveled in center to total distance traveled (P30 - remote)	207
Figure 3.K.: Ratio of distance traveled in center to total distance traveled (P50 - recent)	207
Figure 3.L.: Ratio of distance traveled in center to total distance traveled (P50 - remote)	207
Figure 4.A.: Representative CA1 counting area	212
Figure 4.B.: Representative CA1 c-Fos staining	212
Figure 5.: CA1 c-Fos immunohistochemical analyses	214
Figure 6.A.: Representative ACC counting area	216
Figure 6.B.: Representative ACC c-Fos staining	216
Figure 7.: ACC c-Fos immunohistochemical analyses	218
Figure 8.A.: HPC GluR1 western blot analyses	221
Figure 8.B.: HPC GluR1 western blot representative bands	221

Figure 8.C.: ACC GluR1 western blot analyses	221
Figure 8.D.: ACC GluR1 western blot representative bands	221
Figure 9.A.: HPC GluR2 western blot analyses	224
Figure 9.B.: HPC GluR2 western blot representative bands	224
Figure 9.C.: ACC GluR2 western blot analyses	224
Figure 9.D.: ACC GluR2 western blot representative bands	224
Supplemental Figure 1.A. GluR1 sample western blot	292
Supplemental Figure 1.B. GluR2 sample western blot	292
Supplemental Figure 2. Sex differences in open field task performance	294

Chapter 1

Introduction

1. Overview

Spatial memory is an essential component of survival. It allows for an organism to forage for food and shelter, avoid places that they previously deemed dangerous, and learn to navigate around their environment, all with the use of spatial cues. These dynamic processes are regulated by medial temporal lobe and cortical structures, including the hippocampus and the anterior cingulate cortex, which synergistically incorporate sensory stimuli into a functional “map” that the organism can use to familiarize and orient themselves within their environment. This is mediated through the activation of glutamatergic receptors, which function to strengthen neural synapses, creating robust, long-lasting spatial memory traces. While the underlying behavioral and cellular correlates of spatial memory have been widely investigated in adults, their ontogeny and development in younger organisms are less known.

2. Hippocampal Anatomy

2.1. Functional divisions of the hippocampus

The hippocampus does not work as one cohesive unit, but instead may be functionally parsed into different subunits with various functions. The two major units of the hippocampus are the dorsal and the ventral hippocampus, each with their own distinct input and output connections, and roles in memory processing (Swanson and Cowan, 1977; Moser et al., 1995; Moser and Moser, 1998; Fanselow and Dong, 2010). The dorsal hippocampus receives input

from the caudolateral band of the entorhinal cortex and sends excitatory projections to dorsal parts of the subiculum, presubiculum, and postsubiculum. Additionally, the dorsal hippocampus sends projections to the retrosplenial and anterior cingulate cortices, as well as the caudal and rostral part of the lateral septal nucleus, an area that has been found to regulate social behavior (Fanselow and Dong, 2010; Bredewold et al., 2015). The ventral hippocampus receives input from the rostromedial band of the entorhinal cortex, and sends direct projections to the olfactory bulb, along with bi-directional projections to the amygdala (Saunders et al., 1988; Pitkänen et al., 2000; Cenquizca and Swanson, 2007; Fanselow and Dong, 2010).

The ventral hippocampus has been shown to be involved in emotional memory processes, like fear, and influences the fear memory processing of the amygdala, given that the ventral, but not the dorsal, hippocampus projects directly to the amygdala (Moser and Moser, 1998; Fanselow and Dong, 2010). The dorsal hippocampus contributes to spatial memory processes due to the fact that place fields in this region are more compacted, as opposed to the ventral hippocampus, where the place fields are more dispersed (Jung et al., 1994). A study completed by Moser et al. (1993) supports this hypothesis. After performing localized dorsal hippocampal lesions and testing rats in the Morris water maze, they reported impaired latencies during training, along with a reduction in the amount of time spent in the target quadrant during the probe test. The rats that received lesions in their ventral hippocampus, however, experienced no such impedance in their spatial learning abilities. Based on these results, they concluded that the dorsal hippocampus is more important for the encoding of new spatial information than the ventral aspect (Moser et al., 1993; Riedel and Micheau, 2001). Further evidence to support this hypothesis comes from studies completed by Moser et al. (1995) and Pothuizen et al. (2004), who also reported that spatial learning was impeded if the dorsal portion of the hippocampus was

lesioned. They further noted that dorsal hippocampal lesions were just as effective as complete hippocampal lesions in disrupting spatial working memory, as opposed to lesions of the ventral hippocampus, which resulted in no disruption of spatial working memory.

2.2. Anatomical subregions of the dorsal hippocampus

The dorsal hippocampus consists of three major regions: the parahippocampal gyrus, the dentate gyrus, and the cornu ammonis (CA) fields (Amaral, 1993; Cohen et al., 1999; Lavenex and Amaral, 2000; Wible, 2013).

The parahippocampal gyrus is one of the first structures to receive inputs into the hippocampus, generally from the temporal, frontal, and parietal lobes (Lavenex and Amaral, 2000). It is comprised of areas TH and TF, both populated with pyramidal cells (Insausti et al., 1987; Kobayashi and Amaral, 2007). Area TH is located at the medial aspect of the parahippocampal gyrus and lies adjacent to the presubiculum. It is comprised of an outer lamina, defined by a weak layer II and narrow layer III, and an inner one, defined by a more pronounced layer V and a narrower layer VI, which is fused with layer V (Insausti et al., 1987). Area TF occupies a larger area of hippocampal gyrus and is bordered laterally by area TE and medially by area TH, the presubiculum, and area 29m. Similar to area TH, area TF can be parsed into numerous laminae; a thin layer II, a wide layer III, a prominent layer IV, and densely packed layers V and VI (Insausti et al., 1987). Pyramidal cells in the parahippocampal gyrus synapse with layers I, II, and III of the entorhinal cortex (Insausti et al., 1987; Wellman and Rockland, 1997).

The entorhinal cortex is divided into five layers. The superficial plexiform or molecular layer, referred to as layer I, contains a dense band of fibers with very few neurons. Layer II is the

outermost cell layer and contains “stellate” or “modified pyramidal cells”, which project to different layers of the dentate gyrus (Dolorfo and Amaral, 1998; Canto et al., 2008). Layer III is primarily composed of medium to large sized pyramidal neurons, while the deep border of layer III (also referred to as layer IV) is the cell-sparse fiber layer, known as the lamina dissecans. Finally, layer V is stratified and composed of pyramidal neurons that vary in size depending on how deep they are within the layer; neurons in layer Va are often medium to large sized, while neurons in deeper layers (Vb and Vc) are mainly small and densely packed (Canto et al., 2008).

The dentate gyrus is a well-conserved part of the hippocampus that possesses distinct unidirectional projections to CA3 pyramidal cells (Treves et al., 2008). It is composed of three layers of neurons: the molecular layer, the granular layer, and the polymorphic layer. The molecular layer is the outermost layer and contains dendrites of the dentate principal cells. This layer also possesses axons that originate from layers II and VI of the entorhinal cortex, the ipsilateral hilar mossy cells, and the contralateral hilar mossy cells (Witter and Amaral, 1991; Dalley et al., 2008). The granular layer is aptly named as it is composed of densely packed granule cells, which project and bifurcate close to the soma and molecular layer. The subgranular layer, one of the few areas of the brain where adult neurogenesis occurs, is found between the granular and polymorphic layer, and contains an abundance of progenitor cells (Eriksson et al., 1998; Dalley et al., 2008). The polymorphic layer, commonly referred to as the hilus, is the deepest layer of the dentate gyrus. It is found between the granule cell layer and border of the CA3 dendritic layer. Mossy cells are the most abundant cell type found in the hilus and are defined by the spiny dendrites and thorny excrescences on both their cell bodies and proximal dendritic shafts (Treves et al., 2008).

The CA1, CA2, and CA3 regions are the principal pyramidal cell fields in the hippocampus and are often the focus of research concerned with memory encoding and retrieval (McNaughton and Morris, 1987; Chevalleyre and Siegelbaum, 2010). Although the CA4 region has been distinctly defined, it appears to be part of the polymorphic layer of the dentate gyrus, commonly referred to as the hilus, and possesses mossy cells (Andersen et al., 2007). The CA regions are each composed of layers, or “strata”: the stratum pyramidale, the stratum lacunosum-moleculare, the stratum lucidum, the stratum oriens, and the stratum radiatum. The stratum pyramidale contains the cell bodies of pyramidal cells and various interneurons (Andersen et al., 2007). Pyramidal cell layers in the CA1 are more tightly packed than those in the CA2 and CA3 regions.

The stratum lacunosum-moleculare is the most superficial layer of the hippocampus, where fibers from the entorhinal cortex, the nucleus reuniens of the midline thalamus, along with afferents from other regions, terminate. In the CA2 and CA3 regions, the stratum lacunosum-moleculare receives inputs from layers II and VI of the entorhinal cortex, while the stratum lacunosum-moleculare in the CA1 receives input from layers III and V of the entorhinal cortex (Witter and Amaral, 1991; van Groen et al., 2003). The stratum lucidum, which is found exclusively in the CA3 field between the pyramidal cell layer and the stratum radiatum, contains mossy fiber terminals from the dentate gyrus granule cells (Witter and Amaral, 1991; Andersen et al., 2007). Axons from cells within the layer II of the entorhinal cortex synapse directly with the dendritic spines of granule cells in the dentate gyrus. Mossy fibres are formed by the axons of these granule cells and form synaptic connections with the proximal apical dendrites of pyramidal cells in the stratum lucidum of the CA3 via the hilus of the dentate fascia (Andersen et al., 1971; Sandler and Smith, 1991; Witter, 2007; Neves et al., 2008).

The stratum oriens is a deep, relatively cell-free layer containing the basal dendrites of the pyramidal cells and various interneurons, along with some of the myelinated Schaeffer collateral connections from CA3 to CA1. The stratum radiatum possesses the majority of Schaeffer collateral connections (Andersen et al., 2007). The axons of the CA3 project into the fimbria, but then send the Schaffer collaterals back through the CA3 and travel to the CA1, where they form synapses with the apical dendrites of pyramidal neurons in the stratum oriens and stratum radiatum, which then project to the subiculum (Witter, 2007; Kajiwara et al., 2008; Maccaferri, 2011). Both the subiculum and the stratum lacunosum-moleculare of the CA1 also receive direct projections from neurons in layer III of the entorhinal cortex (Kajiwara et al., 2008; Maccaferri, 2011). Axons of pyramidal neurons of the CA1 project into the septum, hypothalamus, and contralateral hippocampus via the fimbria (Andersen et al., 1971). In addition to receiving input from cortical regions, layer V of the entorhinal cortex receives output from the CA1 via the subiculum, which then projects to other brain regions, such as the mamillary nuclei, the thalamus, and the nucleus accumbens (Amaral, 1993; Wible, 2013).

3. Hippocampal Development

3.1. Morphogenesis of the hippocampus

The hippocampal formation can first be observed during the early stages of embryonic development and develops in a similar pattern in all mammals (Bayer, 1980a). As early as embryonic day (E) 14, the characteristic folds and curvature of the hippocampus begin to form from the neuroepithelium in the dorsomedial wall of the telencephalon, which curves into the lateral ventricle. This curvature continues into E15 and becomes a distinguishing feature of the cortical sheet by E16 (Bayer, 1980a). The most rapid rate of hippocampal growth also occurs

during this time, where hippocampal volume increases by 1900% from E16 to E17. From E18 to E19, the daily rate of growth increases by 66%, and by 29% from E19 to E20. After birth, the volume continues to increase daily by about 26% from postnatal day (P) 1 to P7, and by 12% from P7 to P21 (Bayer, 1980a). In the human fetus, a linear increase in total hippocampal volume has been reported, whereby the average hippocampal volume increases from 0.23 mL at 22 weeks of gestation to 0.60 mL by 30 weeks of gestation (Jacob et al., 2011).

The hippocampus develops from two connected primordia in the telencephalon. A portion of the entorhinal cortex and part of the parasubiculum and presubiculum develop from one primordium in the dorsoposterior wall, while the subiculum, the CA region, and the dentate gyrus develop from another primordium in the dorsomedial wall (Bayer, 1980a).

The entorhinal cortex first begins to develop on E16, when layer I forms in a cell-sparse zone located superficially throughout the primordium (Bayer, 1980a). On E17, a cortical plate develops deep to layer I in the lateral entorhinal cortex and is separated from the neuroepithelium by a transitory zone. On E18, the cortical plate, along with a cell-sparse fibrous zone, appears throughout the entorhinal cortex. On E19, the cortical plate diverges into a central cell-sparse zone, which becomes layer IV, surrounded by superficial and deep cell-rich laminae, which become layers II and III, and layers V and VI, respectively. Layer IV is indistinct due to the many spindle-shaped cells throughout it and layers V and VI (Bayer, 1980a; Ray and Brecht, 2016). From E19 to E22, the laminae become larger, while the neuroepithelium and transitory zones become progressively smaller, until they disappear in the early postnatal period. By E22, cells are added to layer II, layer III is thick and densely packed, and layers IV, V, and VI are distinct (Ray and Brecht, 2016). The parasubiculum and presubiculum are first distinguishable in

the superficial lamina on E22, when the cortical plate ceases to diverge medially and forms the non-bifurcated cortex adjacent to the entorhinal cortex (Bayer, 1980a).

The boundary between the subiculum and the CA region is indistinguishable during the early stages of embryonic development (Bayer, 1980a). Between E16 and E17, the subiculum undergoes the most rapid rate of growth, when a number of cells leave the neuroepithelium region of the subiculum. The growth rate then slows between E19 and E20, which coincides with the rapid increase in volume of the pyramidal layer (Bayer, 1980a). The stratum pyramidale continues to increase in volume from 4% on E18 to 22% at P21 (Bayer, 1980a). After birth, the pyramidal layer decreases in depth yet extends in length, and sharply curves posteriorly while retracting into the dentate gyrus (Bayer and Altman, 1974). The formation of the stratum oriens becomes evident between E17 and E19, when it occupies between 36% and 40% of the total volume. The stratum radiatum and stratum lacunosum-moleculare begin to form by E16, and their volumes increase from 7% to 38% by P21 (Bayer, 1980a).

The dentate gyrus is the last hippocampal structure to appear. The dentate gyrus begins to develop between E18 and E19, where cells from the neuroepithelium of the choroid plexus accumulate in a mass near the outgrowth of the fimbria and form the dentate primordium; at this stage, the primordial volume is called the hilus (Bayer, 1980a). The volume increases from 4% on E18 to 10% on E22, and then gradually decreases by 5% on P21. The outer limb of the granular layer begins to form in the anterior and posterior dentate gyrus on E20, and becomes more evident between E21 and E22, while the molecular layer is not present until P1 (Bayer, 1980a). At birth, the crest and arms of the dentate gyrus form, and between P3 and P5, the endal arm extends further rostrally, separating the proliferative zone from the pial surface (Bayer and Altman, 1974).

3.2. Cytogenesis of the hippocampus

Much like the majority of the morphological development of the hippocampus, most of the neuronal components of the hippocampus propagate during gestation (Semple et al., 2013). Neuron development within the hippocampal system begins within layer IV of the entorhinal cortex. These cells are most heavily labeled by thymidine- H^3 , a radioactive DNA-precursor, by E10. At this time, a few neurons in layers II and III are also visible, but not to the extent of those found in layer IV, while no radioactively-labeled cells are present in layer I. By E12, the density of heavily labeled neurons in layers II, III, and IV has reached its peak. By E14, neuron formation in layers II, III, and IV has virtually ceased, with a few new neurons labeled in layer I (Angevine, 1965). At P0, calbindin-positive pyramidal cells in layer II exhibit a grid-like arrangement similar to that observed in adults (Ray and Brecht, 2016). While these neuronal clusters are visible as early as E18, dendritic clustering is not initially apparent until P4, with adult-like clustering occurring at P8. Calbindin-positive pyramidal cells in layer III are transiently present at E18, and decline progressively until about P20, when their density reaches adult-like levels (Ray and Brecht, 2016). Interestingly, these pyramidal cells exhibit a dorsal-to-ventral maturation profile. Patches of calbindin-positive pyramidal cells express doublecortin, a well-established marker for immature neurons, throughout layer II at P8. At P16, only the ventral patches co-localized with doublecortin, while the dorsal parts exhibited a low degree of overlap. In adults, no co-localization of calbindin-positive pyramidal cells with doublecortin was evident, indicating a delayed functional maturation of these pyramidal cells (Ray and Brecht, 2016).

Neurons within the molecular layer of the dentate gyrus begin to form between E10 and E15, with peak numbers formed by E13, while neurons within the granular layer form over a longer period, from E10 to P20. The granule cells first begin to form between E10 and E11 and

are found near the tip of the suprapyramidal limb. The number of these cells continues to increase along this area through the outer edge of the infrapyramidal limb from E15 until birth (Angevine, 1965). After birth, precursor cells proliferate into the deeper layers of the granular layer, producing the granule cells of both limbs of the dentate gyrus. This pattern of migration indicates that, unlike the stratum pyramidale and other cortical areas, neurons within the granular layer follow an “outside-in” sequence (Angevine, 1965). The peak of granule cell production occurs during the first two weeks after birth. While 25% of the cells in the granular layer have stopped dividing and started to differentiate between P0 and P3, the majority are formed between P4 and P7 while the remaining population of cells continue to form past P16 (Bayer and Altman, 1974). In the molecular layer, a small portion of cells is formed during the first postnatal week, with the bulk of the population forming between P8 and P16 and a small population that have not yet formed by P16 (Bayer and Altman, 1974). While polymorph cells of the hilus and pyramidal cells of the CA4 originate prenatally, most cells within the hilus are formed between P4 and P15, with a small population still to be formed after P16 (Bayer and Altman, 1974). Doublecortin (DCX), a gene that encodes a 40-kDa microtubule-associated protein expressed mainly in neuronal precursors in the developing brain, has been used as a marker for neurogenesis within the dentate gyrus. While this marker is highly expressed in newborns, its immunoreactivity decreases with age after birth (Yoo et al., 2011). These DCX-positive cells have been detected in the dentate gyrus as early as P1 and DCX-positive neuroblasts with short processes formed by P7. By P14, those processes developed and extended in the upper two-thirds of the molecular layer. By P21, DCX-positive neuroblasts were mainly in the subgranular zone, while the density and length of the processes in the molecular layer had decreased compared to P14, suggesting that those cells had begun to mature in that timeframe (Yoo et al., 2011).

The earliest neurons of the hippocampus proper to form are seen on E10 in the stratum pyramidale and are distributed along CA1 and CA2, with a few cells in portions of CA3 (Angevine, 1965). On E11, little has changed in CA1 or CA2 regions, while thymidine-H³-labeled cells begin to appear in the CA3. By E12, both the CA1 and CA3 regions show heavily labeled neurons, with fewer in the CA2. Cytogenesis in the CA3 peaks at E14, while final division in the CA1 continues and maintains its rate in the CA2. By E15, cytogenesis in the CA1 peaks, while neuron formation in both the CA2 and CA3 has ceased. By E18, the formation of neurons in all CA regions is complete (Angevine, 1965; Bayer, 1980b).

Pyramidal neurons continue to develop postnatally. From P5 to P10, pyramidal neurons consist of a long apical dendrite and a few short and somewhat branched basal dendrites with only a few small spines (Pokorný and Yamamoto, 1981). Over that period, the length and number of branches of the basal dendrites and terminal fibers of the apical dendrite increase, with the apical dendrite terminal branch pattern reaching adult values by P10. Between P15 and P24, the lateral branches of the apical dendrite, along with its preterminal fibers, continue to increase, infiltrating the stratum radiatum and stratum lacunosum, respectively (Pokorný and Yamamoto, 1981).

3.3. Development of hippocampal circuitry

Once developed, cells within the various hippocampal regions create a network that is thought to be the source of information processing within the hippocampus and is believed to play a major role in learning and memory (Snyder et al., 1991). This network, commonly referred to as the trisynaptic circuit, consists of three pivotal excitatory synapses which allow for the processing of polymodal sensory information from primary sensory and high-order

association cortices (Stepan et al., 2015). The first synapse in this network begins in the superficial layers of the entorhinal cortex, which provide the main cortical input to the hippocampus (Stepan et al., 2015). Neurons in layer II project their axons to the apical dendrites of dentate gyrus granule cells via the perforant path, while neurons in layer III project their axons to the CA1 and subiculum via the temporoammonic pathway (Witter et al., 2006; Van Strien et al., 2009; Stepan et al., 2015). The dentate gyrus axons give rise to mossy fibres, which go on to innervate pyramidal neurons in the CA3, creating the second synapse in the pathway. CA3 pyramidal cells synapse onto ipsilateral CA1 pyramidal neurons via Schaffer collaterals, thus completing the trisynaptic circuit (Amaral and Witter, 1989; Stepan et al., 2015). Once information has processed through the trisynaptic loop, it returns to the deep layers, specifically, layers IV and V, of the entorhinal cortex from the CA1 and subiculum, where it is then projected to high-order association cortices, such as the cingulate cortex (Stepan et al., 2015).

The formation of the perforant path occurs early in development. Fricke and Cowan (1977) observed the earliest stage of the projection at P3 using injections of ^3H -proline. At this stage, they were unable to distinguish the medial and lateral entorhinal areas but did find evidence of labeling in the stratum moleculare and the granule cells of the medial blade of the dentate gyrus. By P6, the labeling from the entorhinal cortex to the dentate gyrus was clearer, with connections localized into two distinct laminae; over the intermediate one-third of the stratum moleculare and the outer one-third of the stratum moleculare. This observation indicated that, by P6, the general pattern of the entorhinal-dentate gyrus connection had begun to resemble that found in mature animals (Fricke and Cowan, 1977). By P12, the entorhinal projection to the dentate gyrus was more sharply defined and concentrated in the outer portion of the stratum moleculare. This observation lead Fricke and Cowan (1977) to conclude that by P12, the general

organization of the entorhinal input to the dentate gyrus is indistinguishable from that in mature animals.

Much like the perforant path, the mossy fiber pathway connecting the dentate gyrus to the CA region manifests early in development. Amaral and Dent (1981) first observed the formation of the projections at P3, where fascicles of mossy fibers are first seen in the stratum lucidum. By P5, there are a few intra- and infra-pyramidal mossy fibers along the CA region. From P9 to P14, the size and number of the mossy fibers continue to increase in the CA region, with their extensions surpassing the length of those seen in mature animals. Aside from a decrease in length of the mossy fiber extensions, there are few changes to the mossy fiber pathway between P14 and P28, indicating that the mature form of the pathway has largely developed by P14 (Amaral and Dent, 1981).

To assess the development of CA3-CA1 circuitry, Hsia et al. (1998) used electrophysiological approaches to study changes in the organization of synapses during postnatal development. By comparing spontaneous EPSCs recorded before and after application of TTX, they were able to distinguish large numbers of single inputs from CA3 cells onto single CA1 cells. They observed a developmental enhancement of coupling between individual CA3-CA1 cell pairs, whereby the average number of release sites per CA3 cell that released transmitter onto CA1 cells was lowest from P4-P6. From there, those release sites increased from P8 to P13, from P16 to P19, and even into early adulthood, suggesting that this circuitry develops over a longer timeline compared to others within the hippocampus (Hsia et al., 1998). Dumas and Foster (1995) made similar observations, stating that CA3-CA1 synaptic function appears to be immature through the third postnatal week, despite an enhanced LTP (long-term potentiation) magnitude over that time.

Development of the connectivity between the subiculum and entorhinal cortex appears to lag relative to other connections in the circuit, with immature monosynaptic connections first appearing around P7 to P8. After stimulating the parasubiculum at P5 and P6, Koganezawa et al. (2011) observed no postsynaptic potentials in the entorhinal cortex. They were however able to observe the first indications for functional projections from the subiculum to the entorhinal cortex at P7, but only after using strong stimulations. In fact, no measurable signals in the entorhinal cortex in response to bipolar stimulation of the subiculum were observed before P9, with the first depolarizing synaptic responses occurring at P9. During this time, stimulation of the subiculum resulted in signals mainly in layers II and III of the entorhinal cortex. At P10, those signals were noted in layers V and VI as well. From P12 to P14, the responses in both the deep and superficial layers became stronger, with signal sizes in the deepest layers being significantly larger to all other layers (Koganezawa et al., 2011). Little had changed in the signals from P15 onwards, leading Koganezawa et al. (2011) to conclude that monosynaptic connections from the subiculum to the entorhinal cortex reach adultlike levels by around P15.

4. Spatial Memory

4.1. Defining spatial memory

The ability to navigate safely around an environment is paramount to the survival of almost all species and depends on cognitive capabilities required to find and remember locations. All organisms need to be able to leave their dwellings, be it a nest, den, or house, and navigate around an environment for food, to avoid predators, locate mates, and return safely to their original location (Vorhees and Williams, 2014). This capability has been attributed to spatial memory processes, a subtype of episodic memory that stores information within the spatio-

temporal frame (O'Keefe and Nadel, 1978; Sharma et al., 2010). Spatial memory is defined as the storage and retrieval of information required for identification and navigation of proximal and distal space (Mehta, 2014). This is accomplished through the integration and processing of sensory cues throughout the environment that facilitate successful navigation. Spatial memory is influenced by knowledge of the 3-dimensional elements of the environment, referred to as dimensionality, orientation, and self-motion (Burgess et al., 2002).

An organism uses a variety of strategies that incorporate spatial memory and the sensory cues found in the environment to successfully navigate within their surroundings. These strategies include allocentric and egocentric wayfinding. Allocentric spatial processing is characterized by the ability to navigate using distal sensory cues, i.e. cues that are located outside and away from the organism and is dependent on the relational position of objects in space (Mehta, 2014; Vorhees and Williams, 2014). The use of maps is an example of an allocentric frame of reference as they are flexible to landmark changes and allow the organism to make decisions on where things are in space with respect to one another and their location (Ekstrom et al., 2014; Mehta, 2014). When arriving at a landmark or viewing it from a distance, the organism could remember that the destination is halfway between one landmark and another and use this information to navigate their way to the location. Egocentric wayfinding is characterized by the ability to navigate using internal cues; by feedback from limb movements for speed, direction, and sequence of turns, optokinetic flow as the organism moves past surrounding objects, and proximal cues (Vorhees and Williams, 2014). In short, it is a navigation strategy that is related directly to processes generated by the observer (Mehta, 2014). Navigating a route would employ egocentric strategies, given that the signs or cues available are usually used as a marker of where to change direction along the route, but provides no relational information (Mehta, 2014;

Vorhees and Williams, 2014). Unlike allocentric strategies, egocentric navigation can operate with or without the use of visual cues, such as navigating in darkness (Vorhees and Williams, 2014). Given that these two strategies are somewhat contrary in nature, it should come as no surprise that different brain regions mediate their processes. Allocentric navigation has been shown to be heavily reliant on the functioning of the hippocampus, the entorhinal cortex, and surrounding structures, while egocentric navigation has been shown to depend more so on the dorsal striatum and connected structures (Brasted et al., 1997; Burgess et al., 2002; Vorhees and Williams, 2014).

4.2. Spatial memory and the brain

Given the comprehensive nature of spatial memory processes, it should come as no surprise that a number of brain regions play a role in those processes. One such brain region is the parietal cortex. Investigations into the mnemonic information-processing role of the parietal cortex first came about following deficits that emerged in patients with parietal cortex damage. The patients exhibited an inability to discriminate near from far objects, to read or draw maps or diagrams of familiar spatial locations, and a loss of long-term geographic knowledge combined with the inability to form new cognitive maps (DiMattia and Kesner, 1988a). Kesner et al. (1991) trained rats on a cheese board task either before or after lesioning the parietal cortex. They reported that lesioning the parietal cortex resulted in an increased distance traveled to find the correct food location in both rats that were postoperatively trained and tested, and preoperatively trained and postoperatively tested (Kesner et al., 1991). They suggested that optimal performance in tasks such as the cheese board and Morris water maze require both a reference or knowledge-based memory component and a working or data-based memory

component. The latter, of which they attributed proper parietal cortex functioning, emphasizes the operation of a spatial cognitive map strategy or spatial rules. As such, they suggested that the parietal cortex functions to utilize those spatial rules and subserve an allocentric spatial cognitive map (Kesner et al., 1991).

While the contribution of the parietal cortex to spatial memory processes has been found, the anterior and posterior portions of the parietal cortex contribute to those processes in different ways. Save et al. (1992) compared the cognitive and behavioral consequences on an open field task following lesions of either the anterior region of the posterior parietal cortex or the posterior region of the posterior parietal cortex in rats. Neither lesions impaired habituation, which indicated that neither of these regions were involved in encoding of information. After displacing one of the central objects to a new location, Save et al. (1992) reported a nonselective reaction directed at both displaced and nondisplaced objects in rats with lesions to the anterior region; that is to say that the rats indiscriminately reexplored both displaced and nondisplaced objects for the same amount of time. Rats with lesions to the posterior region failed to react to the spatial change. Save et al. (1992) discussed that rats with lesions to the anterior region reacted to the change in the overall geometrical pattern formed by the objects, but could not locate the change, while the deficit induced by lesions in the posterior region indicated a role for this area in spatial information processing. From this, they posited that the anterior region might be required for localization of displaced familiar cues, while the posterior region may be more involved in visuospatial processing (Save et al., 1992).

While Save et al. (1992) provided evidence outlining the involvement of the posterior parietal region in visuospatial processing, the involvement appears conditional in nature and dependent on the attentional requirements of the task. DiMattia and Kesner (1988a) lesioned the

parietal cortex of rats either before or after being trained on two eight-arm radial maze tasks, each varying in the degree of required attention effort. They reported that the rats that received pretraining lesions were unable to acquire either task, while the rats that received posttraining lesions exhibited an impairment only on the task requiring the most attentional effort and showed no decline on the task that required the least amount of attentional effort (DiMattia and Kesner, 1988b). Based on these results, the parietal cortex must be functional prior to learning either task to develop an internal spatial representation of the environment in which it is being tested. Damage to that area hinders the acquisition of novel spatial information. Furthermore, the ability to process complex integration of spatial relations within the immediate environment, that is, to integrate simple spatial stimuli into a composite whole, is highly dependent on the parietal cortex (DiMattia and Kesner, 1988b).

With an understanding of the role the posterior parietal cortex plays in spatial processes, research has focused on the specificity of the representations relying on that region. Studies comparing the involvement of the parietal cortex in egocentric versus allocentric spatial processes have shown that this region seems to play a substantial role in the coding of allocentric spatial processes (Kesner et al., 1989; Save and Moghaddam, 1996; Zaehle et al., 2007). One piece of evidence comes from parietal cortex lesions, which found that damage to that area resulted in impairments in an allocentric spatial localization task, specifically, a cheese board task, while having no effect on an egocentric spatial localization task, specifically, an adjacent arm task in a radial arm maze (Kesner et al., 1989). Rats were able to dissociate baited adjacent from non-baited, non-adjacent arms, but were hindered in their ability to recall the memory of an initial location of food and acquire the memory of a novel location of food (Kesner et al., 1989). While there is a clear involvement of the posterior parietal cortex during both egocentric and

allocentric spatial tasks in humans, the neural circuits mediating each spatial coding strategy are slightly different (Vallar et al., 1999; Galati et al., 2000; Committeri et al., 2004; Zaehle et al., 2007). Specifically, egocentric spatial coding recruits circuits localized mainly within the precuneus, located in the medial parts of the posterior superior parietal cortex, while allocentric strategies activate circuits within the right superior and inferior parietal cortex (Zaehle et al., 2007).

In addition to the parietal cortex, spatial memory processes have been attributed to functions of the frontal cortex (Ragozzino et al., 1998). Sutherland et al. (1982) reported a significant decline in performance on the Morris water maze task in rats that received medial frontal cortex aspiration lesions. The task they employed did not have a working memory nor a temporal ordering component, indicating that the hindered performance was a result of an impairment in spatial mapping ability. They went on to discuss how this deficit is similar to clinical reports of humans with right frontal damage resulting in spatial learning deficits (Sutherland et al., 1982). fMRI studies have been used to provide evidence for the contributions of the prefrontal cortex to spatial memory. Activation within the prefrontal cortex was higher when the participants underwent a spatial memory task compared to nonspatial control tasks (McCarthy et al., 1994).

While fMRI studies have been used to substantiate a role for the frontal cortex in spatial memory processes, they are also effective in elucidating the precise subregions involved in those processes. Much like the spatial memory functions of the parietal cortex, there is evidence to suggest that these processes are localized in particular subregions of the frontal cortex. It appears that activation of the frontal cortex following a spatial memory task is greater and more consistent in the right hemisphere than the left (McCarthy et al., 1994, 1996; D'Esposito et al.,

1998). Pinpointing the precise subregion responsible for spatial memory processing within the frontal cortex has proven to be a bit more challenging, with multiple reports putting forth suggestions for different areas. It was first proposed that the prefrontal cortex is organized into functionally distinct dorsal and ventral regions, with the mid-dorsolateral prefrontal region associated with processing spatial memory and the ventrolateral prefrontal region principally concerned with nonspatial visual processes (D'Esposito et al., 1998; Owen et al., 1998). McCarthy et al. (1994, 1996) reported findings that would corroborate this hypothesis, reporting increased activation in the right middle frontal gyrus (Brodmann's area 46) during a spatial memory task. Jonides et al. (1993), however, employed a different spatial memory task and found significant regional cerebral blood flow in the ventrolateral frontal cortex (Brodmann's area 47). Owen et al. (1996) used five spatial memory tasks, which varied in terms of the extent to which they required different executive processes and found increased activation in either, or both, the ventrolateral and/or the mid-dorsolateral frontal cortex depending on the executive functioning requirement of the task. Given the discrepancy that exists in the literature, both Petrides (1994) and Owen et al. (1996) have put forth a hypothesis that may account for the inconsistencies. They suggested that the dorsal and ventral subdivisions differ in the type of executive processes subserved, rather than the type of information being processed, i.e. spatial versus nonspatial information. Specifically, they posit that the mid-dorsolateral region is involved in active monitoring and manipulation of spatial information within working memory, while the ventrolateral frontal cortex is involved in the organization and execution of a sequence of spatial moves retained in working memory (Petrides, 1994; Owen et al., 1996, 1998; D'Esposito et al., 1998). Evidence for this comes from Owen et al. (1996) study, whereby they found increased activation in the mid-dorsolateral prefrontal cortex during spatial working

memory tasks that require greater monitoring of remembered information than the other spatial memory tasks used, which activated only the ventrolateral prefrontal cortex.

While reports indicate that the parietal cortex may be involved in both allocentric and egocentric processes, the medial prefrontal cortex shows preferential mediation of one process over the other (Kesner et al., 1989). Some of the earliest literature surrounding this notion examined monkeys with frontal and parietal lesions and found that each type of lesion produced impairments on both allocentric and egocentric spatial tasks (Pohl, 1973). Butters et al. (1972) built upon this and examined patients with prefrontal cortical lesions during two different spatial tasks, one requiring the rotation of external objects (allocentric or extrapersonal) and the other requiring personal rotation (egocentric). They reported that patients with prefrontal cortex damage were most impaired on the personal, or egocentric task (Butters et al., 1972). Further studies in rats have corroborated these findings. Aggleton et al. (1995) lesioned the medial prefrontal cortex in rats and tested them on different spatial tasks. They reported marked deficits in the task that taxed more egocentric cues in rats with medial prefrontal cortical lesions, while showing unimpaired performance in more allocentric tasks. They proposed the presence of a direct pathway from the hippocampus to the anterior thalamic nuclei then to the medial prefrontal cortex that mediates the processing of egocentric information, and that the impaired performance observed resulted from the interruption of the flow of information (Aggleton et al., 1995). Contrarily, Kolb et al. (1983) observed significant, yet relatively mild, impairments on the radial arm maze, an egocentric spatial task, along with impaired learning of the Morris water maze, an allocentric spatial task, following lesions of the medial prefrontal cortex. They suggested that, while these results do not provide evidence supporting the view that the medial prefrontal cortex is related exclusively to egocentric navigation, they do suggest that the medial

prefrontal cortex plays a larger role in the control of spatially guided behavior than has been previously reported (Kolb et al., 1983).

4.3. Assessing spatial memory

The fundamental basics of spatial memory capabilities involve the organism learning which location(s) provide safety, food, or some other desirable object using visuospatial cues (Hodges, 1996). The subject generally undergoes multiple trials and is evaluated on factors such as the amount of time it takes to reach the location, the length of the path taken, etc. These assessments are most commonly done with the use of mazes. While the intent of employing these mazes is to assess spatial memory capabilities, a variety of other cognitive processes may inadvertently be tapped, including associative learning, non-spatial memory, temporal order, conditional discrimination, or anxiety (Hodges, 1996). These mazes range from featureless, unconstrained arenas to those with predetermined pathways and restricted routes (Hodges, 1996). They also differ among many dimensions, including the type or availability of spatial cues used, the task requirements which range from spontaneous exploration to complex sequences of choices, and the motivation, which may involve aversive escape, the search for shelter, or novel objects or food at particular locations (Hodges, 1996). The use of visuospatial, associative, and/or sensory cues is also paramount in these tasks, as they provide the tools that the animal depends on to navigate its way through the environment. These cues also determine the navigation strategy used to find a particular location. Tasks that are reliant on allocentric navigation, such as the Morris water maze, depend on distal cues, whereas tasks that require more egocentric navigation depend on proximal and internal cues (Vorhees and Williams, 2014). As such, assessing allocentric functions requires a test environment that has ample distal cues,

but is free of proximal cues, which is one reason why the use of an open pool of water, such as the water maze, has become a principal device to test this strategy (Vorhees and Williams, 2014). Assessing egocentric navigation requires a test environment that provides ample proximal cues while minimizing, or eliminating, distal cues, such that the animal must rely entirely on internal cues. This is generally accomplished by completing the task in complete darkness or by blindfolding/hooding (Vorhees and Williams, 2014). Interestingly, these mazes also detect the search strategies animals use to navigate their way around their environment in search of safe or rewarded locations. In the radial arm maze, for example, some animals always enter adjacent arms, while others choose alternating or opposite arms. In the water maze, animals initially circle around the pool walls, then progressively travel into the inner area of the pool and spend a greater portion of the time in the training quadrant, finally taking a direct path to the platform (Hodges, 1996).

The radial arm maze is one of the tasks employed to assess spatial memory. First developed by Olton and Samuelson (1976), it consists of a central hub with 8 or 12 arms radiating out from the center along with visible distal cues. Before training, the animal is food restricted for motivation, after which they are placed in the center and allowed to collect food pellets from baited arms. During testing, the animal is assessed by which arms they visited once versus those visited more than once, until all baits are obtained. The animal ostensibly uses the spatial cues available to recognize the arms previously visited. Errors are calculated based on the number of visits to empty arms or revisits to previously baited arms (Sharma et al., 2010; Vorhees and Williams, 2014). The training protocol and data interpretation are simple and can be used to assess both spatial working memory and spatial reference memory while inducing minimal stress on the animal (Sharma et al., 2010). Despite the advantages, researchers have

noted some fundamental limitations in the procedure. For one, the animal can use a serial or chaining strategy, that is, entering each arm successively in a systematic order, instead of using spatial cues, and outcomes become difficult to distinguish from working memory (Sharma et al., 2010; Vorhees and Williams, 2014). Another issue is that the maze has proximal and olfactory cues which may serve as strategies to assist in navigation, making it unclear whether distal cues are being used, and making it difficult to dissociate the allocentric from the egocentric spatial strategies employed (Sharma et al., 2010; Vorhees and Williams, 2014). Researchers have used strategies to circumvent this limitation, including allowing the animals to freely explore the maze before the test to saturate the maze with olfactory cues and cleaning and/or rotating the arms while preserving the baited locations (Roullet et al., 1993; Dudchenko, 2004; Sharma et al., 2010). Another limitation stems from the fact that the rates of acquisition in this task are significantly longer compared to other mazes (Hodges, 1996). As such, this task is not well suited to assess drug effects on learning since acquisition is slow, involves multiple cognitive components, and would require the drug to be administered over many weeks (Hodges, 1996).

The Morris water maze is another method used to assess spatial memory function and is in fact the most widely used procedure. First developed in 1981 by Richard Morris, it was designed to ameliorate some of the limitations encountered with the radial arm maze. Instead of a channel-type maze, Morris used a featureless, circular pool of water with multiple distal cues. A submerged platform is provided as reinforcement that was neither close to the wall nor in the center (Morris, 1981; D'Hooze and De Deyn, 2001; Maei et al., 2009; Sharma et al., 2010; Vorhees and Williams, 2014; Gallagher et al., 2015). During training, the animal navigates the pool to find the submerged platform using the distal, visual cues surrounding the pool. The starting point for each trial is in a different location and the animal must use the cues to navigate

the location of the platform from each varying starting point (Vorhees and Williams, 2006, 2014; Maei et al., 2009; Sharma et al., 2010). Initially, the animals exhibit a high degree of thigmotaxis, whereby their swimming is localized around the perimeter of the pool. As learning progresses, they begin to swim out from the edge in search of the platform (Vorhees and Williams, 2014). As training progresses, the latency to find the hidden platform generally decreases, reflecting the implementation of a spatial strategy (Vorhees and Williams, 2006, 2014; Maei et al., 2009; Sharma et al., 2010). Unlike the radial arm maze task, which may require as many as 50 trials to acquire the spatial memory, significant learning can occur in as few as 10 trials with the water maze (Hodges, 1996). While reduced latencies may ostensibly indicate spatial learning has occurred, they may also reflect the adoption of non-spatial strategies, such as weaving and circling that often result in unintentionally finding the platform (Maei et al., 2009; Vorhees and Williams, 2014). To differentiate spatial from non-spatial strategies, a single-trial probe test is given, whereby the platform is removed from the pool and the animal is free to search the area for, typically, 60 seconds. During this time, the animal is evaluated based on their spatial bias for the former location of the platform, that is, the amount of time spent searching within proximity of the goal area (Maei et al., 2009; Vorhees and Williams, 2014).

Different strategies are used by the subject to help them find the hidden platform. The first strategy involves the animal learning the sequence of movements needed to reach the platform, referred to as the praxic strategy. The taxic strategy involves the animal using cues or visual proximal guides to reach the platform. The spatial strategy involves the animal incorporating information about the spatial location of the platform based on the spatial configuration of distal cues to reach the target (Brandeis et al., 1989; Sharma et al., 2010).

Several features of the Morris water maze have proven to be beneficial for assessing allocentric spatial navigation. For one, while rodents are natural swimmers, they prefer to be out of water, providing sufficient motivation to actively search for an escape. The use of water also balances motivation to escape over a variety of differences among groups of animals, which is not influenced by treatment-induced differences in body weight, appetite, or the reward value of the reinforcer, as occurs in appetitive tasks. While appetitive tasks are subject to satiation effects, water continues to be a motivating factor from the first trial to the last (Vorhees and Williams, 2014). Furthermore, the use of water eliminates the possibility that animals use olfactory cues to navigate the maze, ensuring that only allocentric spatial strategies are employed and assessed (Sharma et al., 2010). Another advantage is that no prior preparation, such as food or water deprivation, is required to complete the task, and learning occurs faster on the water maze compared to other mazes, both of which limit the number of days needed to proceed with experimentation (Hodges, 1996; Sharma et al., 2010; Vorhees and Williams, 2014). The results provided by the water maze have been largely accepted as valid and reproducible for investigations focusing on reference and spatial working memory and has been shown to be highly sensitive in the assessment of hippocampal function (D’Hooge and De Deyn, 2001; Dudchenko, 2004; Sharma et al., 2010).

Despite its many advantages, the Morris water maze task does carry with it certain limitations. One disadvantage is the species-specific response characteristics elicited by the water maze in some strains that are not conducive to the task requirements. These include excessive floating or persistent thigmotaxis rather than active searching for the platform. This is especially common in mice, which have been shown to slightly underperform on this task relative to rats (Sharma et al., 2010; Vorhees and Williams, 2014). The most commonly stated

criticism of the water maze is that it imposes stress on the animal. This critique can be interpreted as both an advantage and disadvantage of the task. The neurochemical changes resulting from swimming-induced stress may interfere with testing cognitive function. It may also create variable behavioral outcomes, whereby some animals may react to the stress by frantically and aimlessly swimming around while others freeze, producing inaccurate behavioral results (Sharma et al., 2010; Vorhees and Williams, 2014). However, in moderation, the stress provides the appropriate motivation to incentivize performance required to assess learning and memory. In short, too little as well as too much stress is counterproductive to performance, and midrange levels of stress optimize performance (Vorhees and Williams, 2014). To ensure this, researchers suggest habituating the animal before acute trials are conducted (Morris, 1981; Sharma et al., 2010).

4.4. Spatial memory and the hippocampus

The hippocampal contribution to spatial memory processes has been well documented. One of the first, and most famous, studies linking the importance of the hippocampus to memory storage processes was the report on patient Henry Molaison (H. M.). After a long history of seizures, H. M. underwent a bilateral medial temporal-lobe resection, which ultimately lead to the destruction of the anterior two thirds of the hippocampus and hippocampal gyrus bilaterally (Scoville and Milner, 1957). It was found that H.M. had a complete loss of memory for events 19 months prior to the surgery, along with a partial retrograde amnesia for 11 years leading up to the surgery; his earlier memories remained intact (Scoville and Milner, 1957; Frankland and Bontempi, 2005). After observing the case of H. M., Scoville and Milner (1957) proceeded to collect data from other patients who underwent similar bilateral medial-temporal resections. In

almost all of the patients, most of the areas removed included a portion of the anterior hippocampus and the hippocampal gyrus. They discovered that the patients whose hippocampus was either damaged or removed had severe recent memory deficits similar to those of H. M., while the patients whose hippocampus remained intact experienced no such deficit (Scoville and Milner, 1957). Damage to the hippocampus, be it caused by surgery, disease, or otherwise, impinged on the ability to retain recent memories, while concurrently impeding the ability to form new memories. The memories formed at least 3 years prior to the damage, however, remained unaffected and intact (Scoville and Milner, 1957). These studies suggested that the hippocampus was responsible for the storage and recall of recent memories, i.e. memories formed minutes to hours after encoding, and played a role in mediating the consolidation of recent memories into remote stores, but had little influence on memories that had been previously integrated into remote stores for at least one year (Scoville and Milner, 1957; Rose and Symonds, 1960; Volpe and Hirst, 1983; Zola-Morgan et al., 1986; Nadel and Moscovitch, 1997; Dudai, 2004; Frankland and Bontempi, 2005).

With the use of lesions and pharmacological inhibition, researchers have since built upon Scoville and Milner's findings, providing evidence that proper hippocampal functioning is a necessity for spatial memory processing. Aggleton et al. (1986) lesioned the hippocampus and subjected rats to a spatial memory task. They reported that the lesions resulted in severe impairments in a spatial alternation task compared to the control animals. Aggleton et al. (1986) then compared their performance on the spatial task to a non-spatial memory task. They reported that lesions that impaired spatial memory did not impair the acquisition of a non-spatial task; that is, they were able to learn and perform the task at normal rates (Aggleton et al., 1986). These results show a clear dissociation of the two processes in terms of hippocampal contribution and

indicate that the hippocampus is required for spatial memory processes. Hippocampal lesion also resulted in a significantly longer latency and pathlength to find a hidden platform in the Morris water maze, along with the inability to use spatial relational representations of the environment to discriminate between different food locations (Morris et al., 1982; Lavenex et al., 2006; Tzakis and Holahan, 2016).

In humans, equivalent behavioral phenotypes can be seen in those with hippocampal damage. Patients with unilateral hippocampal atrophy showed impaired spatial memory in a nine-box maze task, while retaining object working memory capabilities. Furthermore, these spatial impairments were correlated with volumetric measures of the hippocampus and surrounding structures, and not with measures of the remaining temporal cortex (Abrahams et al., 1999). Astur et al. (2002) tested patients with unilateral hippocampal resections on a virtual analogue of the Morris water maze and compared their performance to that of patients with extra-hippocampal resections. They reported an impairment in spatial navigation only in patients with hippocampal resections, whereby they were unable to use the spatial cues in their environment to navigate to the hidden platform (Astur et al., 2002).

As previously mentioned, of the two spatial strategies (egocentric and allocentric), the hippocampus appears to be more so involved in allocentric navigation processes. After lesioning the hippocampus in rats and testing them in the Morris water maze, Morris et al. (1982) reported impairments in the ability to find the hidden platform using distal cues during training and testing. These same lesions, however, did not impair the ability to find the platform using brightly colored proximal cues placed directly above the platform. These results provide support for the notion that the hippocampus is involved in allocentric-based, but not egocentric-based navigation (Morris et al., 1982; Nadel, 1991; Ekstrom et al., 2014). O'Keefe et al. (1975)

compared performance on an allocentric (place learning) task and egocentric (cue learning) task after lesioning the fornix of rats, a major afferent/efferent pathway of the hippocampus. While the lesions did not hinder successful completion of the task when cue learning was required, they severely impaired performance when place learning was required. Taken together, these results show a clear indication that proper hippocampal functioning is required for allocentric, but not egocentric, tasks (Nadel, 1991).

4.5. The hippocampus and the cognitive map theory

The hippocampus is clearly involved in mediating spatial memory processes. The question then becomes: what attributes does the hippocampus possess that makes it such a valuable structure to spatial memory processes? O'Keefe and Nadel (1978) provided some insight into this question, offering up their theory of the existence of a cognitive map within the hippocampus. The term "cognitive map" was first coined by Tolman (1948), who used it in reference to a wide range of capacities in the rat, including the ability to learn about spatial relationships in an environment, commonly known as place learning. When this theory surfaced, the general consensus about memory was that there was an integrated memory function, subserved by a centralized neural system (Nadel, 1991). This single memory unit was assumed to be localized to a select few regions and was involved in integrating all sorts of information. What many researchers at the time failed to recognize was that H.M. and other patients did not exhibit this "global" deficit in memory, but rather retained some forms of learning (Nadel, 1991). It was this notion that led to the inception of the cognitive map theory. This theory posits the existence of a neural cognitive map within the hippocampus that functions to provide the necessary information required for place learning.

Place learning is learning to use any available route to approach or avoid a particular location within an environment, independent of specific stimuli originating from the target location (O'Keefe et al., 1975). This neural map is supposedly made of two systems: a place system and a misplace system. The place system is a collection of cells that represent the spatial relations amongst features of an environment and allows the individual to orient and locate themselves within that environment (O'Keefe et al., 1975). The misplace system is a collection of cells which signals changes within the environment; that is, they signal mismatches or inconsistencies between what is perceived and what was previously stored in memory relating to the environment (O'Keefe et al., 1975). Both the place and misplace system allow an organism to explore and orient itself within a familiar environment, while building maps of novel environments and incorporating new information into existing maps (O'Keefe and Nadel, 1978). Additionally, the misplace system also encourages exploration within the environment. O'Keefe and Nadel (1978) suggested that exploration is the resulting behavior that occurs once the misplace system detects an inconsistency within the environment, and, in the absence of the hippocampus, all forms of this behavior should, hypothetically, be abolished. They went on to elaborate that exploration forms the substrate for maps within the environment, and that the information gathered through exploration establish the cognitive maps within the hippocampus (O'Keefe and Nadel, 1978; Nadel, 1991).

Furthering the concept of the existence of a cognitive map within the hippocampus, O'Keefe and Dostrovsky (1971) identified single units in the hippocampus that fired when a rat was in a particular environment. These single units came to be known as place cells, and the particular place in the environment that elicited a response from these cells was referred to as the place field, which was defined by the configuration of objects in the environment (O'Keefe and

Dostrovsky, 1971; Hodges, 1996). The location of the place field of a place cell remained stable in a familiar environment, even after being removed from that environment over an extended period (Muir and Bilkey, 2001). The place cells have been reported to elicit activity only when the organism is oriented toward the specific stimulus that was encoded; replacing that stimulus in the same direction produced no cellular response (O'Keefe and Dostrovsky, 1971; Hodges, 1996). It has also been suggested that the impaired performance in spatial tasks, like the water maze, observed in organisms with hippocampal damage is a result of the loss of these place cells. According to O'Keefe and Dostrovsky (1971), without the hippocampus, and its place cells, the organism would no longer be able to incorporate the cues found in their surroundings to facilitate exploration within their environment, and the brain would ultimately lose any information stored within these spatial maps, leading to an inability to perform on spatial tasks.

Evidence for the existence of these place cells and their importance in spatial memory processing has been put forth in various studies. After training rats on various reward-seeking place learning tasks to perform at a high level, O'Keefe and Speakman (1987) performed a probe trial, whereby the animal was exposed to the training environment, but not permitted to enter the body of the maze. Only after the cues were all removed were they allowed to explore the maze. Units that had stable place fields in the environment were measured during this probe trial to assess whether these places cells would retain their place specificity (O'Keefe and Speakman, 1987; Nadel, 1991). As the animals navigated around this familiar environment, now devoid of cues, the place cells continued to fire in the specific locations, allowing the animal to seek the reward in the correct location. When they mistakenly entered the wrong location, the place cells also fired incorrectly (O'Keefe and Speakman, 1987; Nadel, 1991). They concluded that the cognitive map represented memory of the environment incorporated with the animal's

movements, which allowed for the updating of the place-field representation, and the resulting correct navigational behavior (O'Keefe and Speakman, 1987; Nadel, 1991).

Jones-Leonard et al. (1985) reported similar findings after training rats in a radial arm maze in which place cells had been identified. The place field locations remained the same, with the exception of one circumstance, whereby the room lights were turned off either before or after the animal had been exposed to the environment during that session. Turning the lights off before the rat was exposed to the environment resulted in little to no place cell activity within the place field that had previously elicited a strong response. Turning the lights off after the rat was exposed to the environment resulted in maintained place cell activity within the correct locations, even though the rat may not have been in the place field the moment the lights went out (Jones-Leonard et al., 1985; Nadel, 1991). From these results, they deduced that the spatial selectivity of place cell activity is a result of the animal's memory of the spatial relationships of the cues within their environment (Jones-Leonard et al., 1985; Nadel, 1991). These works, along with others provided the neural basis for visuospatial navigation coupled with the understanding that the hippocampus builds cognitive maps to provide a flexible allocentric strategy for spatial navigation (Hodges, 1996).

4.6. Cellular underpinnings of spatial memory

With a solid understanding of the role the hippocampus plays in spatial memory function, the next question becomes: what are the cellular mechanisms that mediate spatial memory processes? One mechanism is through the activity of the abundant glutamatergic receptors found within the hippocampus. *N*-Methyl-*D*-aspartate receptors (NMDAr) are a subtype of ionotropic glutamate receptors whose involvement in spatial memory processes within the hippocampus has

been extensively studied. The pore of this voltage-gated channel is blocked by a Mg^{+2} ion, which requires a sufficient depolarization event to be displaced (Fleischmann et al., 2003). Following depolarization of the cell, and subsequent release of the Mg^{+2} ion, the pore opens to allow Ca^{+2} ions to enter the cell, creating an influx which triggers the induction of LTP, a crucial component to synaptic strengthening and memory processes. The influx also creates a cascade of intracellular signaling events, which lead to the activation of immediate early genes, such as c-Fos, which function to regulate synaptic protein synthesis (Fleischmann et al., 2003; Tzakis and Holahan, 2016).

Shapiro and Caramanos (1990) assessed NMDAr to hippocampal function and learning and memory. They administered MK-801 to antagonize NMDAr prior to subjecting rats to a water maze task. They reported that NMDAr antagonism resulted in impaired acquisition of the task and attributed that impairment to an ability to induce hippocampal LTP (Morris et al., 1989; Shapiro and Caramanos, 1990). The same findings have been confirmed with the use of other NMDAr antagonists, suggesting that NMDAr function is crucial to the acquisition of spatial memories (Stringer et al., 1983; Monaghan et al., 1989; Nakazawa et al., 2004).

In addition to spatial memory acquisition, NMDAr also seem to contribute to the consolidation of spatial memories. Initial investigations into the role of NMDAr in spatial consolidation examined the effects of infusing NMDAr antagonist 1 or more days after training on a spatial memory task. To their surprise, they found that the same doses of the drugs that were shown to impair spatial memory acquisition had no effect on learning if administered after training. Naturally, they concluded that NMDAr were required for memory acquisition, but not for consolidation (Morris et al., 1990; Norris and Foster, 1999; Nakazawa et al., 2004). Packard and Teather (1997a, 1997b) revisited these findings and shortened the timeframe of NMDAr

antagonist administration to a few minutes following training on a Morris water maze then tested them on the following day. They reported spatial memory impairments during the probe trial on the following day. They offered the suggestion that the NMDAr-dependent time window for consolidation was less than 2 hours following completion of the training (Packard and Teather, 1997a, 1997b).

Despite its involvement in both spatial memory acquisition and consolidation, evidence suggests that NMDAr do not play a role in spatial memory retrieval. Liang et al. (1994) infused AP5 into the hippocampus prior to a probe test on the Morris water maze. They reported that pretest infusion of AP5 had no effect on spatial memory retrieval (Liang et al., 1994). There is evidence that NMDAr antagonism has little effect on α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-mediated fast synaptic transmission. Assuming, therefore, that NMDAr blockade could completely abolish the existing pattern of synaptic weights within the hippocampus, AMPAr function would still allow for cells to fire and transmit the established network pattern. This would likely explain the omission of NMDAr functioning in spatial memory retrieval processes (Collingridge et al., 1983; Martin et al., 2000; Martin and Morris, 2002; Nakazawa et al., 2004).

While NMDAr mediate the slow, delayed postsynaptic response, AMPAr, another ionotropic glutamate receptor subtype, are responsible for the fast, immediate postsynaptic response to glutamate release (Riedel et al., 2003). As previously mentioned, once NMDAr are activated, they signal the induction of a series of intracellular signalling cascades that act as secondary messengers. These second messengers include cAMP-dependent protein kinase A (PKA), cAMP-dependent protein kinase C (PKC), and calcium/calmodulin-dependent protein kinase II (CAMKII), all of which go on to phosphorylate different serine/threonine residues on

AMPAr. Phosphorylation of AMPAr increases their sensitivity to glutamate, potentiates the responses of the channel, which ultimately facilitates synaptic plasticity and their related cognitive functions (Greengard et al., 1991; McGlade-Mcculloh et al., 1993; Riedel et al., 2003; Tzakis and Holahan, 2016).

Compared to the overwhelming amount of data outlining the detailed understanding of NMDAr function and their contribution to a variety of memory processes, investigations into AMPAr function have been relatively scarce. One reason for this is that pharmacological AMPAr agonists are extremely difficult to use in vivo given their excitotoxic nature. Antagonizing AMPAr, on the other hand, prevent fast excitatory transmission, which essentially mimic the effect of a local anaesthetic or a γ -aminobutyric acid (GABA) agonist. The resulting behavioural effects is more so a representation of structural silencing or inactivation and outlines the involvement of that brain region but not the specific contribution of AMPAr within that region. Additionally, AMPAr antagonism impairs postsynaptic depolarisation, which reduces the activation of NMDAr (Riedel et al., 2003).

Of the four subunits that compose AMPAr (GluR1-4), evidence has shown that the GluR1 and GluR2 receptor subunit play the most prominent role in cognitive processes. GluR1 is directly phosphorylated by CAMKII, PKC, and PKA, which potentiates AMPAr function. Increased phosphorylation has been associated with the continued expression of LTP, indicating a role in the strengthening of synapses and the memory trace following its acquisition (Reisel et al., 2002; Riedel et al., 2003; Kessels and Malinow, 2009; Huganir and Nicoll, 2013). To test if GluR1 phosphorylation is, in fact, necessary for learning and memory, Lee et al. (2003) generated and tested a line of mutant mice that lacked the two major GluR1 phosphorylation sites serine 831 (Ser831) and serine 845 (Ser845), substituting them for alanine. They reported

that this knockin mutation prevented phosphorylation of GluR1, inhibiting both LTP and long-term depression (LTD). In terms of cognitive capabilities, these mice showed normal acquisition on a Morris water maze task; that is, their performance in finding the hidden platform improved with each successive training trial and was no different from the performance of their wild-type counterpart. Interestingly, their probe trial performance also showed no indication of impaired spatial memory retention, since they spent more time in the target quadrant following a short 2-4-hour delay (Lee et al., 2003). They then increased the delay between training and testing to as much as 24 hours, and surprisingly, found that the mutant mice were no longer spending greater than chance time in the target quadrant, indicating that their spatial memory retention was impaired (Lee et al., 2003). Bernabeu et al. (1997) reported that the levels of the GluR1 subunit increased within the first 3 hours after training. Together, these findings suggest that GluR1 activity is necessary for memory formation during the first few hours following acquisition and show clear involvement of the GluR1 receptors in spatial memory consolidation.

The GluR2 subunit performs a more regulatory role in AMPA function and normal brain function. GluR2 confers many of the major biophysical properties of the receptor, including receptor kinetics, single-channel conductance, and Ca^{+2} permeability, and influences receptor assembly and trafficking (Isaac et al., 2007). The majority of GluR2 mRNA exist in an edited form, changing the amino acid sequence from a neutral glutamine to positively charged arginine at position 607, rendering any heteromeric GluR2-containing AMPAR Ca^{+2} -impermeable (Tanaka et al., 2000; Henley and Wilkinson, 2016). This Ca^{+2} -impermeability results in a subunit possessing low single-channel conductance and a linear, outwardly rectifying current-voltage relation, while channels lacking or containing unedited versions of GluR2 are Ca^{+2} -permeable, and possess a higher channel conductance with an inwardly rectifying current-voltage relation

(Burnashev et al., 1992a, 1992b; Lerma et al., 1994; Jia et al., 2001; Isaac et al., 2007). However, AMPAR present in mature neurons either lacking GluR2 or containing unedited GluR2 are Ca^{+2} -permeable and are essential to synaptic plasticity and NMDAR-independent learning and memory processes, not to mention prevent excitotoxicity, neuronal death, and pathogenicity (Tanaka et al., 2000; Kwak and Kawahara, 2005; Wright and Vissel, 2012; Henley and Wilkinson, 2016).

Unlike GluR1, which is essential for LTP induction and expression, GluR2 is not required to induce LTP. Rather, it is involved in another form of synaptic plasticity, known as homeostatic synaptic scaling. This form of plasticity functions to scale synaptic transmission through a negative feedback mechanism. It resets the neuronal firing rate and has been suggested to counteract LTP to maintain network stability and prevent runaway excitation. In essence, it provides necessary negative feedback signaling while preserving the encoded information within the individual synapses (Turrigiano et al., 1998; Gainey et al., 2009; Siddoway et al., 2014). Further investigations into the GluR2 subunit and its variants have provided greater insights into the contribution of GluR2 to memory processes. Kollekter et al. (2003) reported on a GluR1-independent form of synaptic plasticity within the hippocampus mediated by C-terminal splice variant of the GluR2 subunit, GluR2(long). While GluR1-lacking mice failed to elicit LTP, their hippocampal circuitry and spatial reference memory remained intact, suggesting a form of LTP-inducing synaptic plasticity that is not dependent on GluR1 (Zamanillo et al., 1999; Kollekter et al., 2003). This form of plasticity was mediated by GluR2(long) trafficking, which was shown to maintain about 35% of the steady-state AMPAR-mediated responses and was responsible for 50% of the resulting potentiation within the CA3-CA1 synapses following LTP induction (Kolleker et al., 2003). Furthermore, GluR2-deficient mice showed impaired spatial and non-spatial learning performance in the water maze, along with several other aspects of abnormal

behavior, suggesting that impaired GluR2 transmission leads to deficits associated with the hippocampus, among other brain regions (Gerlai et al., 1998).

Given this lack of understanding and fragmentation of knowledge compared to NMDAr, it should come as no surprise that a growing interest into these receptors and their role in memory formation has occurred. After administering the AMPAr antagonist CNQX within the dorsal hippocampus of rats prior to training on a water maze task, Liang et al. (1994) reported impaired spatial memory acquisition, i.e. the latency to find the platform was significantly longer compared to their control counterparts. These same impairments were reported in other investigations that employed an AMPAr antagonist prior to acquisition of a spatial memory task, while others reported an enhancement in acquisition following administration of an AMPAr agonist prior to training. These results ostensibly suggest that AMPAr are crucial to the acquisition of spatial memories (Granger et al., 1993; Cain et al., 1996; Filliat et al., 1998; Riedel et al., 1999; Micheau et al., 2004; Tzakis et al., 2016). This, however, is not necessarily the case. While they do provide evidence that AMPAr activation may be necessary for spatial memory acquisition, they also may reflect the aforementioned sensorimotor or cellular deficits resulting from AMPAr blockade (Riedel et al., 2003; Tzakis et al., 2016). Therefore, while there is evidence to suggest that AMPAr play a role in spatial memory acquisition, it has not yet been conclusively determined.

Newly acquired memories induce changes in both the density and binding of AMPAr, which produce learning-induced increases in excitation within the neuronal pathways, allowing for the formation and strengthening of the memory trace (Riedel et al., 2003). Within 2 hours post-acquisition, both the density of novel AMPAr and the affinity of their binding sites increased in the CA1 region of the hippocampus, making it apparent that AMPAr are involved in

memory consolidation processes (Tocco et al., 1991, 1992; Cammarota et al., 1995; Bernabeu et al., 1997; Riedel et al., 2003). Jerusalinsky et al. (1992) reported that bilateral infusion of AMPAr antagonist CNQX within the dorsal hippocampus following training resulted in impaired memory retention on a probe test given 24 hours later. These impairments occurred whether CNQX was administered within 0, 90, or 180 minutes following training, suggesting a sensitive time period during which AMPAr activation is necessary for memory consolidation and retention (Jerusalinsky et al., 1992). Micheau et al. (2004) reported similar results following administration of an AMPAr antagonist after the final day of water maze training, whereby the rats spent less time in the target quadrant during a probe test given 16 days later. Consistent with this finding, Tzakis et al. (2016) reported that posttraining administration of an AMPAr antagonist impaired spatial memory retention in rats when they were tested on a water maze task 3 weeks after training. This impairment, however, was not observed following each successive day of training, indicating that the procedural and sensorimotor aspects of the task were not affected by AMPAr blockade, and that the consolidation of newly acquired spatial memories is dependent on AMPAr functioning (Tzakis et al., 2016).

Expression of previously acquired memories appears to be heavily reliant on proper AMPAr functioning. Riedel et al. (1999) reported an impairment in memory retrieval during a probe test after administering an AMPAr antagonist 1 hour prior to the test. While the rats exhibited the appropriate search strategy, they failed to execute it in the target location. These findings suggest that proper AMPAr functioning is necessary to retrieve spatial information about the environment, but unnecessary to retrieve the swimming strategy (Riedel et al., 1999). This finding has been replicated by numerous studies examining the effect of AMPAr antagonism on memory retrieval, indicating a general consensus on the involvement of AMPAr

functioning during memory retrieval processes (Liang et al., 1994; Szapiro et al., 2000; Bast et al., 2005; Tzakis et al., 2016). Bast et al. (2005) observed that AMPAR blockade reduced fast excitatory transmission at perforant-path dentate gyrus, and possibly other intrinsic synapses within the hippocampus, and this reduction in excitatory synaptic transmission may account for the impaired memory retrieval. This effect appears to be long-standing. Bianchin et al. (1993) observed impaired memory expression after intrahippocampal infusions of CNQX before a probe test that was conducted 20 days after training, indicating that proper AMPAR functioning is required for memory retrieval up to at least 20 days following its acquisition.

4.7. Hippocampal development and the emergence of spatial memory processes

Given the evident contribution of the hippocampus to spatial memory processes, the question then becomes: does the emergence of spatial memory processes coincide with hippocampal development? During neural development, the hippocampus undergoes a high degree of connectivity-based changes that have been shown to influence spatial memory processes. One such change is the increase in neurogenesis within the dentate gyrus, which peaks between P15 and P18 (Altman and Das, 1965; Bayer, 1980b; Zhang et al., 2009; Curlik et al., 2014; Tzakis et al., 2016). These granule cells begin to form connections with pyramidal neurons within the stratum lucidum of the CA3 region via mossy fibers from P18 to P20. By P24, the projections proliferate into the distal stratum oriens of the CA3, where they remain stable into adulthood (Holahan et al., 2006). Mossy fiber projections to the CA3 tend to establish their synaptic contacts within the apical stratum lucidum, with sparse contacts in the basal stratum oriens (Ben-Ari and Represa, 1990; Ramírez-Amaya et al., 2001). Ramírez-Amaya et al. (1999)

substantiated the importance of this mossy fiber synaptogenesis in spatial learning by reporting a significant increase of mossy fiber terminals in the stratum oriens of the CA3, which was induced by 3 days of water maze training. Undertraining (training for only 1 day) or overstressing the rats resulted in no such increments of mossy fiber terminals within the CA3, indicating that this synaptogenesis is related to the formation of spatial memory (Ramírez-Amaya et al., 1999). Ramírez-Amaya et al. (2001) then compared the degree of synaptogenesis while impairing acquisition of a spatial memory via NMDAr blockade. NMDAr blockade prior to acquisition resulted in impaired performance during the probe trial, and a subsequent lack of Timm's staining within the stratum oriens, indicating that mossy fiber projections had not proliferated into that layer. NMDAr blockade following acquisition resulted in both normal performance on the water maze probe test and an increased Timm's staining within the stratum oriens (Ramírez-Amaya et al., 2001). They concluded that mossy fiber synaptogenesis is related to remote spatial memory formation (Ramírez-Amaya et al., 2001).

This blocked outgrowth resulting from NMDAr antagonism has also been reported by Holahan et al. (2007), who found that application of an NMDAr antagonist from P17 to P20 inhibited the proliferation of mossy fiber projections into the stratum oriens. They concluded that the propagation of mossy fiber terminals, along with the target selection of those terminals depend on NMDAr regulation of input-dependent processes during development. Furthermore, pharmacological insults administered during this developmental timepoint create long-lasting changes in the hippocampal remodeling process that persist into adulthood, as is evident by the lack of proliferation at P40 following NMDAr blockade from P17 to P20 (Holahan et al., 2007).

Given the correlational relationship between mossy fiber synaptogenesis and spatial memory, it can be hypothesized that the emergence of spatial memory processes coincides with

the period for hippocampal development. Keeley et al. (2010) trained rats from P16 to P26 on the Morris water maze. They reported moderate improvements from P16 to P19, with dramatic improvement on the task from P19 to P20, where the daily latencies began to significantly decrease. These improvements continued on into the last day of training on P26 and had not been observed prior to P20 (Keeley et al., 2010). Furthermore, they observed shorter latencies to locate the platform when retrained on the task 14 days following their last training day (P40) compared to P40 rats that were naïve to the task. These cognitive improvements paralleled the hippocampal axonal remodeling. The stratum oriens:stratum lucidum ratio of synaptophysin staining was larger in the rats that were trained from P16-26 compared to that rats that had initially been exposed to the water maze at P40. These results indicate the existence of a period from P16 to P21, during which time the hippocampus undergoes neurodevelopmental changes and extensive axonal remodeling that coincide with the emergence of spatial function (Keeley et al., 2010).

Further research into the ontogeny of spatial navigation behaviors in rats has yielded similar and consistent results, confirming the overlapping timelines of both hippocampal remodeling and the emergence of spatial processes (Rauch and Raskin, 1984; Wartman et al., 2012; Comba et al., 2015). Rudy et al. (1987) reported on the emergence of both proximal- and distal-cue-based behaviors in rats. They provided evidence to suggest that rat pups of at least 19-days-old were capable of using proximal orientation cues to navigate to the platform, while 17-day-old pups showed no indication of such capabilities (Rudy et al., 1987). They surmised that the platform was not visible to these younger pups and given that their swimming behavior had not yet fully matured, could impede their ability to use proximal cues to navigate the maze. After raising the platform slightly above the surface of the water, they found that the performance of

the younger pups improved, suggesting that pups as young as 17-days-old have acquired the ability to use proximal cues to navigate the maze, provided that the cues can be seen (Rudy et al., 1987). In terms of distal-cue navigation, Rudy et al. (1987) reported little improvement in escape latencies in 18- and 19-day-old pups compared to 21- and 23-day old pups. Additionally, the 19-day-old pups spent an equivalent amount of time in all four quadrants during a probe test, while the 21- and 23- day-old pups spent over 50% of their search time in the target quadrant. They concluded that 19-day-old rat pups are unable to use distal cues to locate the platform and that the ability to do so emerges sometime between 21- and 23-days-old, after the emergence of the ability to use proximal cues (Rudy et al., 1987). Chapillon and Roullet (1996) further confirmed these results in mice and concluded that the capability of using both proximal- and distal-cue-based navigation is evident as early as 22-days-old, given that their performance at that age parallels, and even surpasses, the performance of adult mice. Guskjolen et al. (2017) further assessed the developmental timeline of spatial memory retention capabilities by training and testing mice on a spatial memory task from P15 to P150. They reported that spatial navigation capabilities emerged as early as P15, as evident by a decrease in swim pathlengths over the three training days for each age group. Performance on the probe test, however, suggests a different developmental pattern for the retention of that spatial information. While P15 and P17 mice spent a greater amount of time in the target quadrant 24 hours following their last day of training, they were not as selective 30 days following training. After P20, however, mice retained the acquired spatial information for at least 30 days, suggesting that, while the ability to acquire spatial memories is evident as early as P15, the ability to retain that information remotely may not appear until after P20 (Guskjolen et al., 2017).

Comba et al. (2015) attempted to link the ontogeny of spatial memory capabilities with the developmental changes within the hippocampus. Their behavioral findings were consistent with the literature. They reported shorter latencies to locate a hidden platform in the Morris water maze task for 20-day-old rats compared to 16- and 18-day old rats, suggesting the emergence of spatial function by P20. After examining the development of mossy fiber connectivity patterns, they reported developmental changes within the mossy fiber terminal field distribution. Specifically, they observed an increase in synaptophysin staining within the stratum oriens of the CA3 from P16 to P20, with the greatest staining observed at P20. They concluded that these changes in mossy fiber terminal distribution may support the emergence of spatial behavior by shifting input away from inhibitory interneurons, reducing the number of synapses on inhibitory interneurons within the stratum lucidum, which may lead to an increase in excitatory input on CA3 pyramidal neurons (Comba et al., 2015). Given that these changes in presynaptic mossy fiber terminals are dependent on neural activity that occurs during development, they also examined c-Fos activation within the hippocampus. They reported increased c-Fos activation within the dentate gyrus and CA1 at P20 compared to the younger age groups. They suggested that this increased network activity may facilitate the emergence of spatial behaviors. Furthermore, they reported an increase in labeling within the dentate gyrus and CA3 region at P18 compared to P16, which may provide the necessary increase in neural activity required for the developmental changes in mossy fiber connectivity.

4.8. The fate of newly acquired spatial memories

What is the fate of spatial memories after acquisition? Does the hippocampus simply function as a repository for recent memories, after which it is no longer needed for the

expression of these memories? Or is it constantly involved in all processes involving that memory trace? And if hippocampal contribution is transient in nature, what region takes over the remote processing of these memories? These questions have initiated a myriad of investigations that have produced the formation of three main hypotheses surrounding the neural underpinnings of remote spatial memory: the cognitive map theory, the standard consolidation theory, and the multiple trace theory. Remote memory involves the creation of traces in remote memory circuits mediated by short-term processes that can last between seconds to a few days. Traces that outlast these processes form the basis of a remote memory (Dudai, 2004; Frankland and Bontempi, 2005; Moscovitch et al., 2006). The one common characteristic that all hypotheses agree upon is that the hippocampus receives and integrates information initially registered in the sensory cortices, and from that, forms a memory trace consisting of an ensemble of interconnected hippocampal-neocortical circuits. These hypotheses diverge when it comes to the fate of these memory traces as they gradually become remote.

The cognitive map theory first described by O'Keefe and Nadel (1978) has already been discussed. In short, it proposes that the hippocampus represents the immediate environment of an individual, their location within that environment, and the sensory cues contained within that environment. These spatial representations provide the context in which episodic events are embedded (O'Keefe and Nadel, 1978; Burgess et al., 2002; Moscovitch et al., 2006; Spiers and Maguire, 2007). This hypothesis does not distinguish between cognitive maps acquired recently or remotely. According to the cognitive map theory, the hippocampus is constantly involved in the retention and expression of spatial memories, whether they were acquired recently or remotely. As such, any form of hippocampal damage should result in impaired spatial memory regardless of when it was acquired (Moscovitch et al., 2006).

Nadel and MacDonald (1980) trained rats on a radial arm maze after lesioning the hippocampus and reported an impairment in performance that they attributed to the hindered place learning aspect of the task. Even after the rats underwent at least twice the number of retraining trials postoperatively that they had required preoperatively, they were still unable to acquire and express the spatial memory (Nadel and MacDonald, 1980). Bolhuis et al. (1994) trained rats on a water maze task and then lesioned their hippocampus either 3 days or 14 weeks following acquisition and tested them two weeks after surgery. The rats that received the lesions performed at chance on both retention intervals compared to the sham controls. Lesioned rats also exhibited severely impaired memory during the probe test, while the sham rats showed facilitated learning, suggesting a reactivation of memory rather than relearning (Bolhuis et al., 1994). They concluded that the hippocampus was required for spatial memory retrieval, and that lesioning the hippocampus impaired memory consolidation at all time intervals (Bolhuis et al., 1994).

Skeptics of this hypothesis reference studies showing that the hippocampus is required for the acquisition of spatial information, but remote spatial memories are shown to survive large hippocampal lesions (Teng and Squire, 1999; Rosenbaum et al., 2000, 2005; Moscovitch et al., 2006). These studies have provided evidence that extra-hippocampal regions, and not the hippocampus, may be required for spatial memory expression. Patients with damage exclusively to the hippocampus retain the ability to navigate throughout their environment and perform well on a variety of spatial memory tests. Conversely, lesions to regions such as the parietal, parahippocampal and posterior cingulate or retrosplenial cortex have been reported to impair navigation on spatial tests (Aguirre and D'Esposito, 1999; Moscovitch et al., 2006). Given that these structures share reciprocal, anatomical connections with each other and the hippocampus, it

is possible that the hippocampus is involved in integrating information from these spatial networks with information from other sources. Thus, the hippocampus would serve to facilitate the ability to express recent or remote spatial memories by providing detailed memory representations, rather than being directly involved in their remote storage (Cammalleri et al., 1996; Rockland and Van Hoesen, 1999; Lavenex et al., 2002; Suzuki and Amaral, 2004; Moscovitch et al., 2005, 2006).

The standard consolidation model posits a series of prolonged processes that occur at both the cellular and systems level that function to strengthen the memory trace and could last months or even decades (Moscovitch et al., 2006). Cellular consolidation involves the synthesis of new proteins that strengthen synapses and stabilize memory representations through persistent modifications in synaptic transmission (Debiec et al., 2002; Rudy and Sutherland, 2008; Mednick et al., 2011; Tzakis and Holahan, 2016). The process occurs during and immediately after memory acquisition, at which point the activation of glutamatergic receptors activates a series of intracellular signal transduction cascades, including the cyclic adenosine monophosphate (cAMP) cascade following the activation of adenylate cyclase. From there, isoforms of cAMP-response element binding proteins (CREBs) are phosphorylated by cAMP-dependent kinases, which allow for the modification of cAMP response element (CRE)-regulated genes. These genes include transcription factors that regulate expression of genes whose products are induced after a few hours and are known as late-response genes (Dudai, 2004; Tzakis and Holahan, 2016). Synaptic protein synthesis also induces modifications of the synapse by tagging the activated synapse, attracting new proteins from the cell through a reorganization of the synapse. These new proteins strengthen synaptic tagging and act as retrograde messengers to inform the nucleus of the modification (Frey and Morris, 1997, 1998).

Regulation of gene expression within the nucleus also provides the tagged synapses with newly synthesized messenger RNAs and proteins, further enhancing the specificity of the consolidation process (Dudai, 2004; Tzakis and Holahan, 2016).

Consolidation at the systems level involves a series of processes that ultimately modify the memory trace from being hippocampus-dependent to hippocampus-independent. The initial involvement of the hippocampus, and the neocortical regions that it projects to, is exclusive to the storage and recovery of the memory trace. The initially weak memory trace is stored and strengthened within the hippocampal-neocortical network, with the hippocampus initially possessing the stronger trace (Rudy and Sutherland, 2008; Wang and Morris, 2010; Mednick et al., 2011). The trace is activated and reactivated following memory recall, strengthening the existing neocortical memory. This strengthening is a result of cellular consolidation-mediated modifications of neocortical connections or through the establishment of new connections within the neocortex, after which point the memory trace is now integrated with pre-existing memories within the neocortex (Dudai, 2004; Frankland and Bontempi, 2005). In short, the hippocampus is involved in the ‘teaching’ of information stored in the neocortex, after which point the trace degrades, leaving space for the rapid learning of new information without disturbing the memory stores within the neocortex (Dudai, 2004; Wartman et al., 2012).

According to this model, spatial memories become independent from the hippocampus over time, and as such, hippocampal lesions should result in amnesia for events prior to the lesion, while functional neuroimaging should show diminished activation of hippocampal activity over time (Moscovitch et al., 2006). The case study of patient E.P. provided evidence for the standard consolidation model. E.P. was infected with a virus that lead to encephalitis, which resulted in bilateral hippocampal damage and subsequent amnesia. As a result, E.P. was unable

to recall the spatial layout of his neighborhood, to which he moved following his infection. He was, however, able to describe the spatial layout of the neighborhood he grew up in over 50 years prior to his infection (Teng and Squire, 1999). These results suggest that the hippocampus is required for the acquisition of remote spatial memories, but not for the retrieval of remote spatial memories (Teng and Squire, 1999; Spiers and Maguire, 2007). Comparable findings have been reported in amnesiac patients with extensive hippocampal damage (Rosenbaum et al., 2000, 2005). fMRI studies have shown an increase in hippocampal blood flow during recall of recent news events (within the past 3 years) compared to older news events (Smith and Squire, 2009; Wang and Morris, 2010). Takashima et al. (2006) also reported reduced hippocampal activity when participants were asked to recall memories that were 90 days old compared to those that were 1 day old. Opponents to this hypothesis question whether the memories retained by amnesiac patients are as vivid and detailed as they would be in healthy individuals and argue that the hippocampus may be somewhat necessary in the expression of these memories, if only to provide detailed memory representations (Frankland and Bontempi, 2005).

The multiple trace theory has elements of both the cognitive map and standard consolidation theories. According to this model, the hippocampus is involved in the encoding of information and binds neocortical neurons that represent that information into a memory trace, much like the standard consolidation model. A distributed network of hippocampal neurons encodes this information, after which it functions as an index to the neurons in which the attended information is encoded. This bound ensemble of hippocampal-neocortical neurons forms the memory trace of an episode and represents the memory of the consciously experienced event (Nadel and Moscovitch, 1997; Moscovitch et al., 2005). Once these memory traces are recalled, they are re-encoded into the hippocampal-neocortical network, resulting in the

formation of multiple traces mediated by bundles of hippocampal-neocortical neurons (Moscovitch et al., 2006). The creation of this new trace allows for old memories to be represented by more or stronger traces within the network, making them less susceptible to disruption from brain damage relative to more recent memories (Moscovitch et al., 2005). There is no prolonged strengthening of memory traces within the neocortex and the formation and consolidation process lasts on the order of seconds to, at most, days (Moscovitch et al., 2005). In this respect, the multiple trace theory is reminiscent of the cognitive map theory, and deviates from the standard consolidation model, as the memory trace will always be dependent on the hippocampus to retain its vividness and detail, regardless of when it was acquired (Moscovitch et al., 2006). Based on this hypothesis, the extent and severity of retrograde amnesia resulting from hippocampal damage is dependent on the age of the memory, along with the extent and location of the damage. Hippocampal damage would also hinder the vividness of recollection, impairing the ability to remember detailed spatial information or to richly re-experience the environment, while leaving general semantic-like spatial information intact (Moscovitch et al., 2005, 2006; Spiers and Maguire, 2007).

Evidence largely favors the multiple trace theory over the consolidation model, though it is still not conclusive (Moscovitch et al., 2006). Maguire et al. (2006) reported a case study conducted on patient T.T., a taxi driver in London who, following a rare form of limbic encephalitis, was left with bilateral hippocampal damage, and amnesia. T.T. was able to recognize London landmarks, make proximity and distance judgments, locate places on a London map, and show spared direction-pointing (Maguire et al., 2006; Spiers and Maguire, 2007). Despite these intact spatial functions, he was impaired when it came time to navigate a virtual, interactive environment; he took significantly longer routes and was sometimes unable to

reach his destinations. These impairments were not all-or-nothing; he was error-free on some routes and extensively impaired on others. One significant variable stood out from the others: if the route was composed of a high number of minor, side streets, then he was impaired, yet he was able to successfully navigate using main roads. Since main roads are generally used more often than side streets, and are thus experienced more often, they may have acquired a semantic-like status, becoming independent of the hippocampus (Maguire et al., 2006; Spiers and Maguire, 2007). The side streets, however, are less traveled, more numerous, and are comprised of a complex and dense layout. As such, they require a more detailed representation of topography, which, according to the multiple trace theory is dependent on hippocampal function. Thus, the hippocampal damage incurred by T.T. resulted in a loss of, or inability to access, fine-grained as opposed to coarse spatial representations (Maguire et al., 2006; Spiers and Maguire, 2007).

Rosenbaum et al. (2000) provided further evidence for the multiple trace theory following their investigation on patient K.C. K.C. presented with widespread hippocampal damage following a closed head injury that resulted in amnesia. Like T.T., K.C. exhibited no impairments on a variety of spatial memory processes; he was able to describe alternate routes to a location when the most direct route was blocked, judge the proximity between locations, and order landmarks in the same sequence they would be passed when navigating the area (Rosenbaum et al., 2000; Spiers and Maguire, 2007). His spatial impairments manifest when it came to sketching landmarks of his neighborhood, demonstrating geographical knowledge, and recognizing superfluous neighborhood landmarks (Rosenbaum et al., 2000; Spiers and Maguire, 2007). They concluded that he showed impaired remote memory for detailed spatial information and argued that the results support the prediction from the multiple trace theory that memory for

detailed spatial memory is impaired following hippocampal damage (Rosenbaum et al., 2000; Spiers and Maguire, 2007).

5. Remote Memory

5.1. Memory storage in the anterior cingulate cortex

Once memories are received and processed in the hippocampus, they undergo a process whereby those recently encoded memories solidify and stabilize in the brain in order to persist, a process known as memory consolidation. Memory consolidation involves two levels of processing: the cellular level, which involves regulating gene expression and protein synthesis in localized circuits through intracellular signaling cascades, and the brain systems level, which involves more gradual changes in brain structures spanning over a much longer time frame (Einarsson and Nader, 2012; Wartman et al., 2014). The remote memory consolidation process is mediated by various intricate and complex neural connections between the cingulate cortex, the entorhinal cortex, and the hippocampus (Insel and Takehara-Nishiuchi, 2013).

The anatomical connectivity between the hippocampus and anterior cingulate cortex may support the transfer of memories initially encoded by the hippocampus for remote storage in the anterior cingulate cortex. One pathway that that may support this is the superficial layer of the entorhinal cortex that receives input from the sensory and association cortices and projects to areas of the hippocampus and the cingulate cortex. From there, the hippocampus and the cingulate cortex send output to the deep layer of the entorhinal cortex, which then projects back to the sensory and association cortices (Insel and Takehara-Nishiuchi, 2013; Takehara-Nishiuchi, 2014). A more direct pathway between the hippocampus and cingulate cortex has also been observed, whereby neurons in the ventral CA1 region project to the rostral cingulate, while

neurons in the dorsal CA1 region project to the retrosplenial granular A cortex, a posterior region of the cingulate cortex (Insel and Takehara-Nishiuchi, 2013). The subiculum has the same projections as those in the CA1 region, while also projecting to the retrosplenial granular cortex B, a more anterior, caudal region of the cingulate cortex. Activation of both pathways simultaneously has been shown to strengthen synaptic connections in both the cingulate cortex and the entorhinal cortex, which in turn increases the capacity for remote memory storage capabilities (Insel and Takehara-Nishiuchi, 2013).

Following the medial temporal-lobe resection, H.M. was unable to retain any of his recent memories, suggesting that the hippocampus played a role in the acquisition and retention of recent memories. He was, however, able to remember his earlier, remote memories, those that were encoded several years prior to his surgery. This indicates that there is some hippocampus-independent mechanism that allows for the retention, storage, and reconsolidation of “remote” memories. To examine this hypothesis, (Izquierdo et al., 1997) trained rats in an inhibitory avoidance task and tested them after 1 day, 30 days, or 60 days. They used CNQX to antagonize AMPA receptors in the hippocampus, the amygdala, the entorhinal cortex, or the parietal lobe prior to testing. They found that AMPA receptor blockade in all four regions at the 1-day interval impeded performance, while blockade only in the entorhinal and parietal cortices after 30 days affected performance. After 60-days, however, only injections in the parietal cortex impeded performance, suggesting that, initially, the memories were stored in and retrieved from the neocortex and hippocampus, however as the time increased after learning, the neocortex sustained retrieval on its own (Izquierdo et al., 1997; Meeter and Murre, 2004).

In a follow-up to these findings, Bontempi et al. (1999) infused a (^{14}C)2-deoxy-glucose tracer into mouse brains to assess metabolic activity in different brain regions over time during a

spatial working memory task. They discovered an inverse relationship between the training and testing interval and metabolic activation in the hippocampus. As the training and testing interval increased, metabolic activity in the hippocampus decreased. This indicated that the hippocampus was no longer needed to recall those memories. Conversely, metabolic activity in other cortical areas, specifically the anterior cingulate cortex, increased during recollection of those memories, suggesting that the remote storage and retrieval depended on activity in that region (Bontempi et al., 1999). Damage to the cingulate cortex has also proven to impede the retention/retrieval of remote memories, further demonstrating a role of the cingulate cortex in the remote memory storage process (Insel and Takehara-Nishiuchi, 2013; Wartman et al., 2014).

During the early stages of recent memory formation, spine density on hippocampal cell dendrites increases, allowing for rapid synaptic rearrangement. It has been demonstrated that these same structural changes, specifically dendritic spine growth, develop in the anterior cingulate cortex during remote memory acquisition (Restivo et al., 2009). In mice, recent memory formation was associated with an increase in spine density on hippocampal neurons, but not on cortical neurons. During remote memory formation, however, the inverse was seen, where the spine density on anterior cingulate cortical neurons, but not on hippocampal neurons, increased (Restivo et al., 2009). These differences in temporal changes suggest that the formation and expression of remote memories is a result of gradual structural changes in hippocampal-cortical network connectivity (Restivo et al., 2009). Taxing the demand placed upon the hippocampus expedites the rate of synaptic and systems consolidation processes. After rats underwent training on two hippocampal-dependent spatial tasks, Wartman and Holahan (2014) reported increased dendritic complexity in neurons within the anterior cingulate cortex and the hippocampus at both a recent and remote timepoint relative to rats trained on one spatial

task, or none at all. Specifically, there was a greater degree of branch density and length, along with a greater number of spines and spine density, on both apical and basal dendrites in the anterior cingulate cortex and the hippocampus of rats that were trained on two hippocampal-dependent spatial tasks compared to those trained on only one, or not trained on any behavioral tasks at all. The increased dendritic complexity in this group was even significantly greater at a recent timepoint compared to control rats at a remote timepoint, which suggests that the rate of consolidation in the anterior cingulate cortex is accelerated to account for the increase of incoming spatial information to the hippocampus (Wartman and Holahan, 2014).

Activation of c-Fos can be used as a marker of neuronal activity and as evidence that functional changes have occurred in the anterior cingulate cortex with the expression of remote memories. Teixeira et al. (2006) assessed the retention of spatial memory in mice either 1 day (recent test) or 30 days (remote test) after their last day of training on the Morris water maze. They found that the mice in both groups spent more time in the area associated with the platform compared to other areas, indicating that mice in both groups retained that memory. They compared their behavioral results to c-Fos expression in the anterior cingulate cortex and found there to be significantly higher c-Fos expression in the remote test group compared to the recent test group, suggesting that the anterior cingulate cortex was preferentially involved in the retrieval of remote spatial memories (Teixeira et al., 2006). An increase in c-Fos expression has also been reported in the anterior cortex following multiple, recently-formed hippocampal-dependent memories (Wartman and Holahan, 2013). The pattern of c-Fos activation observed at the recent timepoint resembled the pattern of staining generally observed in remotely-tested groups and was different from that seen following the formation of a single, recently-formed hippocampal-dependent memory. This would suggest that taxing the demand placed upon the

hippocampus expedites the rate at which cortical network recruitment is seen (Wartman and Holahan, 2013).

Inactivation of the anterior cingulate cortex also impairs the expression of remote spatial memories. Mice treated with infusions of lidocaine directly into their anterior cingulate cortex showed no difference in time spent in the area associated with the platform versus other areas during a remote (30 days post-training) probe test using the Morris water maze (Teixeira et al., 2006). This disruption in performance was not seen in mice that underwent the same pharmacological treatment but were tested more recently (1-day post-training), which would indicate that the anterior cingulate cortex plays an important role in the storage of remote, but not recent, spatial memories and its inactivation impedes the expression of those memories (Teixeira et al., 2006). Wartman et al. (2014) reported similar findings after training rats on a radial arm maze task and/or a water maze task and then inactivating the anterior cingulate cortex prior to a probe test given 37 days following the last day of training. They observed a performance deficit during the probe test, the extent of which depended on the processing demand of the hippocampus; rats trained and tested on both the radial arm maze and water maze tasks showed a more substantial impairment in performance relative to those trained and tested on a water maze task only. They suggested that remote spatial memories become more reliant on the anterior cingulate cortex when hippocampal processing demands are increased (Wartman et al., 2014).

5.2. Ontogeny of remote memory capabilities

Investigations into remote memory storage capabilities have predominantly focused on adults, with little information as to when they emerge during earlier developmental stages. While the exact developmental timeline is somewhat ambiguous, investigators have attempted to offer

some insight into when these remote memory capabilities emerge. (Foster and Burman, 2010) tested the emergence of remote fear memory of an environment by training rats on a contextual fear conditioning task on P17, and then testing that memory either 1 day or 1 week following the training day. They reported that the rats exhibited significant fear when tested a week later, but not 24 hours later, indicating that the ability to acquire and consolidate a context-based fear memory, and to form fearful associations, is evident by P17, while the ability to use a contextual representation in a fearful association has not yet emerged. They posited that the associative mechanisms continue to develop between P18 and P23, such that the hippocampal connections to the amygdala and other relevant brain structures had not yet fully developed by P17 (Foster and Burman, 2010). Akers et al. (2012) trained mice on a contextual fear memory task at one of three different developmental timepoints: infancy (P15), adolescence (P30), or adulthood (P60). The rats were then tested at 1, 7, 14, or 28 days following training to assess remote memory storage. They reported that mice trained on P15 exhibited little or no freezing in the presence of a cue following delays of more than one day, suggesting the ability to transfer the recent fear memory into remote stores had not yet developed at this age. Mice trained on P30 or P60, however, showed high levels of freezing for at least 28 days following training, indicating that the emergence of remote memory processes emerged at least by P30 (Akers et al., 2012). Taking results from both studies into consideration, a developmental timeline can be hypothesized, whereby the ability to process recent memories into remote stores develops sometime between P17 and P30 (Foster and Burman, 2010; Akers et al., 2012). Though there are few reports assessing spatial memory function at longer time intervals, Tzakis et al. (2016) provided some preliminary evidence regarding the emergence of remote spatial memory stores. They reported that, when unimpaired by pharmacological insults, rats trained on a water maze task at P18

managed to retain the memory for the hidden platform location up to 3 weeks following their last training day, suggesting that remote spatial memory processes may emerge as early as P18 (Tzakis et al., 2016). Further investigations comparing those processes at different increments of development are required to provide a more accurate timeline for the precise emergence of remote spatial memory capabilities.

6. Current Thesis

6.1. Objectives and hypotheses

Objective 1: Investigate the factors that influence the processing of juvenile-acquired spatial memories from a remote memory standpoint during a crucial period of hippocampal development.

Late postnatal development marks a critical period for hippocampal development, whereby the hippocampus undergoes a series of structural and morphological changes that are thought to facilitate memory processes. This hippocampal remodeling has been hypothesized to mediate the transition from juvenile-like to adult-like remote memory processing. However, the time during this period at which adult-like remote memory processing emerges has yet to be established. To assess this, juvenile rats were trained and tested either recently or remotely on the Morris water maze during a critical period of hippocampal development, and their performance was compared to adult rats. Additionally, massed-type water maze training was compared to spaced-type training to determine whether training type facilitates the transition to adult-like capabilities. Neuronal activation (c-Fos) was quantified within the hippocampus and anterior cingulate cortex to determine the pattern of activation during remote testing for each age group and correlated with their performance on the water maze.

Hypotheses and predictions

It was hypothesized that performance on the spatial task and remote retrieval will improve as the hippocampus becomes more fully developed, reaching adult-like levels near the end of this hippocampal developmental timeframe. The processing and remote storage would likely be enhanced by a spaced-training protocol in the early developmental time points. It was also hypothesized that more advanced hippocampal development would lead to better recall of remote spatial memories, which would come to rely on ACC activity and less on hippocampal activity, as evident by elevated c-Fos labeling in the ACC and reduced c-Fos labeling in the hippocampus following a remote spatial memory test.

Objective 2: Assess the developmental variations in expression of AMPAr subunits GluR1 and GluR2 within the hippocampus and ACC during the critical period hippocampal remodeling and compared to that seen in adults.

Understanding the development of spatial memory abilities requires insight about the development of cellular underpinnings that mediate the transition from juvenile-like to adult-like spatial processing capabilities. The cellular components of interest include AMPAr subunits GluR1 and GluR2, which are involved in the activation of signaling cascades that mediate memory processes. Western blotting was performed on the ACC and hippocampus of rats at developmental ages before, during, and after the critical period of hippocampal remodeling and compared to adults. These analyses will provide insight into any variations in AMPAr subunit expression during this critical period that may further the understanding into the synaptic changes that occur as a result of this remodeling, and when their expression stabilizes into

adulthood. Additionally, immunohistochemistry was performed on the hippocampus in order to further delineate the changes in AMAPr subunit expression that may occur over time.

Hypotheses and predictions

It was hypothesized that expression of the GluR1 and GluR2 subunits will vary as a function of increased hippocampal remodeling. That is, we expected to see an increase in expression of both subunits during this critical period, reaching adult levels during the late phase of hippocampal remodeling, or shortly thereafter. Furthermore, we expected to see immunohistochemical labeling that parallels the degree of expression observed at each developmental timepoint from the Western blotting analyses.

Objective 3: Investigate the hippocampal remodeling-mediated transition from juvenile- to adult-like remote memory processing, and the cellular underpinnings that influence that transition.

Furthering the investigations performed in Objectives 1 and 2, Objective 3 aimed to assess the developmental processing of juvenile-acquired remote memories during a critical period of hippocampal development, and how this remodeling facilitates the transition to adult remote memory processing. Furthermore, GluR1 and GluR2 activity was determined to ascertain the variation in expression as result of hippocampal remodeling and correlate this variation to performance on the spatial task. To do this, juvenile rats were assessed during a period of hippocampal remodeling in an open field task, where they were allowed to explore a novel environment surrounded by spatial cues. They were then be tested at either a recent (24 hours) or remote (3 weeks) timepoint in the same environment to determine the extent to which they recognize it as being familiar using the spatial cues provided. Their performance was compared

to that of adults to determine when during the period of hippocampal remodeling do juveniles process remote memories similar to adults. This objective also attempted to remedy any potential confounds encountered by the water maze task used in Objective 1, such as swimming abilities and fatigue.

Hypotheses and predictions

Upon initial exposure to a novel environment, rats show increased exploration, denoted by the total distance traveled throughout the trial. Ostensibly, as they familiarize themselves with the environment, their inclination to explore decreases, such that re-exposure to that same environment will result in a shorter total distance traveled. As such, we hypothesized a net reduction in total distance traveled between training and testing trials as a function of increased hippocampal remodeling. Furthermore, we expected to see “impaired” performance, i.e. an increase in total distance traveled during testing, in younger ($> \sim P24$) rats as the interval between training and testing increases, furthering the notion that they have yet to acquire the ability to process memories into remote stores. GluR1 and GluR2 levels are expected to correlate with performance on the spatial task, with increased expression in rats who have acquired the ability to consolidate the acquired memories into remote stores, and the greatest variation in expression expected to occur within the ACC at each age point.

Chapter 2

Factors That Influence the Processing of Juvenile-Acquired Spatial Memories in Rats During Hippocampal Development

Nikolaos Tzakis, Matthew R. Holahan
Department of Neuroscience

Manuscript submitted to: Neurobiology of Learning and Memory

Abstract

Postnatal, preadolescent development is characterized by a reorganization of connectivity within and between brain regions that coincides with the emergence of more complex behaviors. The hippocampus is one such region that undergoes extensive postnatal remodeling including neuron proliferation, synaptogenesis and dendritic arborization. As this process of remodeling continues, spatial memory processing functions have been shown to emerge. The current work investigated whether preadolescent spatial memories acquired during this period of hippocampal synaptic modification persist beyond 24 hours and stabilize into the postadolescent period. Rats were trained on the Morris water maze at different time frames from postnatal day (P) 18-26. Testing occurred at either a recent (24 hours) or remote (3 weeks) timepoint. Performance of preadolescent rats was compared to postadolescent rats (P50). Postadolescent rats (P50) showed asymptotic spatial learning within the 3-day training period and showed intact retention at 24 hours and 3 weeks. Spaced training in the P18 group did not improve acquisition or retention at either 24-hour and 3-week testing intervals. The P22 and 24, but not P20, groups showed asymptotic spatial learning within the 3-day period but none of these groups showed intact retention 3 weeks after training. The P18 and P50 groups tested at 24 hours showed more CA1 hippocampal c-Fos labeling than groups tested at 3 weeks. The P50 group tested at 3 weeks showed elevated c-Fos labeling in the anterior cingulate (ACC) compared to the P18 group tested at 3 weeks and the P50 group tested at 24 hours. Spaced training in the P18 group was associated with elevated c-Fos labeling in the ACC at the 3-week test. Groups trained at P20, 22, and 24 showed more c-Fos labelling in the ACC than in the CA1. Results suggest that while spatial information processing emerges around P20, remote spatial retention and the neural substrates that support retention are not in place until after P26. While the ability to process recent spatial

information was evident at P18, it was not until the later period of development (P26) that the ability to strengthen a labile memory engram into remote storage started to emerge.

Keywords: Spatial memory; hippocampus; ACC; development; massed training; spaced training

1. Introduction

The consolidation of spatial memories into remote storage may rely on changes in network connectivity such that there is a gradual disengagement of hippocampal function and a gradual strengthening of cortical connections (Wiltgen et al., 2004; Frankland and Bontempi, 2005, 2006). The animal literature has supported the view that after initial information processing, hippocampal networks become less prominent, while intra-cortical connections come to represent a memory (Bontempi et al., 1999; Frankland et al., 2004; Maviel et al., 2004; Teixeira et al., 2006; Lopez et al., 2012). The processing of multiple hippocampal-dependent memories can accelerate the speed of cortical recruitment such that spatial remote memories come to rely more fully on cortical networks when hippocampal processing requirements are increased (Wartman and Holahan, 2013, 2014; Wartman et al., 2014).

One cortical region hypothesized to be involved in remote memory storage is the anterior cingulate cortex (ACC). Evidence for this comes from investigations showing increased activity and structural changes within the ACC following remote memory testing (Bontempi et al., 1999; Teixeira et al., 2006; Weible et al., 2012). Inactivation of the ACC results in poor performance on remote spatial memory tests (Wartman et al., 2014). These data suggest that the initial spatial memory representation may be acquired, processed, and stored for a short period within the hippocampus, after which it is encoded into remote stores in the ACC.

During hippocampal development, there is an increase in neurogenesis within the dentate gyrus (DG) between postnatal day (P) 15 and 18 (Altman and Das, 1965; Bayer, 1980; Zhao et al., 2006; Curlik et al., 2014). From P18 to 20, mossy fiber connectivity from DG granule cells to CA3 pyramidal neurons shows widespread connectivity changes so that by P24, these projections show adult-like connectivity patterns (Amaral and Dent, 1981; Gaarskjaer, 1985,

1986; Holahan et al., 2006, 2007). These morphological changes coincide with improved performance on spatial memory tasks (Comba et al., 2015). It is hypothesized that, as a result of this extensive hippocampal remodeling, hippocampal-cortical connections are strengthened, facilitating the remote processing and storage of spatial information.

The main objective of this study was to determine the system dynamics that mediate recent and remote spatial memory processing in juvenile rats during a time when the hippocampus undergoes remodeling. The goal was to assess whether juvenile spatial processing and remote storage resembled that of adults and to investigate the contribution of both the hippocampus and ACC to recent and remote processing of spatial information.

To further assess the ability of juvenile rats to store remote spatial memories, two different water maze training procedures were used: massed and spaced training. Spaced training was defined as a longer intertrial interval (~5-8 minutes between each trial) within a day compared with massed training where no intertrial interval was given. The work of Commins et al. (2003) demonstrated the beneficial effects of spaced training in adult rats. In that investigation, spaced-trained rats had lower escape latencies during acquisition relative to massed-trained rats and spent significantly more time in the platform area 7 days after acquisition compared to the massed-trained rats. Other investigations have reported similar benefits of spaced training (Bello-Medina et al., 2013; Bercovitz et al., 2017; Vlach et al., 2019). The present study examined whether remote memory storage of spatial information acquired during the juvenile period could be facilitated by using a spaced training procedure.

To compare recent and remote memory, a probe test was given either 24 hours or 3 weeks following water maze training. To assess the contribution of the hippocampus and ACC to these memory stages, immunohistochemical examination of c-Fos was used to label recently

activated neural populations. It was hypothesized that performance on the spatial task and remote retrieval would improve as the hippocampus became more fully developed, reaching adult-like levels near the end of this hippocampal developmental timeframe. The processing and remote storage would likely be enhanced by a spaced-training protocol in the early developmental time points. It was also hypothesized that more advanced hippocampal development would lead to better recall of remote spatial memories, which would come to rely on ACC activity and less on hippocampal activity, as evident by elevated c-Fos labeling in the ACC and reduced c-Fos labeling in the hippocampus following a remote spatial memory test.

2. Materials and methods

2.1. Subjects

Male Long-Evans rats (LERs) were used throughout the experiment. Rats were born in the temperature and humidity-regulated animal facility at Carleton University. The day the pups were born was marked as P0. Pups were weaned on P18. Newly weaned rats were provided with solid food pellets soaked with some water and placed in a ceramic dish in the cage, along with a dish containing fortified water gel. Unsoaked food pellets were also placed in the cage. For all experiments, rats were group-housed in polycarbonate cages with a 12-hour light-dark cycle: lights on 8h00, lights off 20h00 with food and water provided *ad libitum*. Animal care was conducted in accordance with the Canadian Council on Animal Care (CCAC) guidelines and approved by the Carleton University Animal Care Committee.

2.2. Apparatus

Water maze

The pool was opaque, white polypropylene with a submerged platform 2 cm below the water surface. Water temperature averaged $25\pm 1^{\circ}\text{C}$ throughout behavioral testing. The water maze was a white, circular, polypropylene pool measuring 124 cm in diameter, 31 cm in height and filled with water to a depth of 25 cm. The platform was made of clear Plexiglas and measured 11 cm in diameter. Three 6' tall banners with black shapes (circles, squares, or triangles) on a white background were placed near the outer edge of the pool at different sides and were present during all trials. Rat movements in the pool were tracked using the HVS Image 2100 Tracking System (HVS Image, Buckingham, UK). The water was quickly cleaned after each trial and the pool was drained and refilled every other day.

2.3. Water maze training

All subjects were given eight, one-minute trials each day over three training days. Each trial began from a different start point on the perimeter of the pool and the same sequence of start locations was used each day. The platform remained in the same quadrant for all eight trials during each of the three training days. Latency and pathlength to reach the platform were recorded for all trials. If an animal did not reach the platform within 60 seconds, they were guided to it. After each trial, the animals remained on the platform for 15 seconds before being removed, dried, and placed back into the water maze for the next trial. Following the final trial on each day, every animal was dried with a towel and placed in another holding cage on a heating pad in the housing room for 15 to 20 minutes before being returned to their home cage.

2.4. Water maze testing

The rats underwent a probe test in the water maze at a certain time following their last training day. Each rat was subject to a single 60-second trial, and each trial began from a different start point on the perimeter of the pool. For all probe tests, the platform was removed from the water maze. The amount of time spent in the different quadrants and predefined platform locations were recorded for all trials. After each probe test, the rats were returned to their home cage. Each rat was then injected with 1 ml sodium pentobarbital one hour following their probe test until they were no longer responsive to a toe pinch. The rats were then transcardially perfused with 50 ml 0.9% sodium chloride, followed by 50 ml 4% paraformaldehyde and their brains collected.

2.5. Conditions

To compare memory acquisition, consolidation, and retention capabilities of juvenile and adult rats, rats were separated into 4 different groups. Each group differed in the age at which the rats were trained and tested on the water maze. Rats in the juvenile:recent group ($n = 7$) were trained from P18-20 and tested at P21. Rats in the juvenile:remote group ($n = 9$) were trained from P18-20 and tested at P41. Rats in the adult:recent group ($n = 9$) were trained from P50-53 and tested at P54. Rats in the adult:remote group ($n = 7$) were trained from P50-53 and tested at P74.

To determine whether a different water maze training procedure would facilitate remote spatial memory processing, P18 rats were either massed-trained or spaced-trained on the water maze. Rats that were massed-trained underwent each of the 8 trials in successive order on each training day. Rats that were spaced-trained were given an inter-trial interval between each trial

until all rats in the group completed the same trial then proceeded to the next trial until all 8 trials were completed. Rats were tested either 24 hours or 3 weeks following their last training day. Rats in the massed:recent group (n = 10) were massed-trained from P18-20 and tested at P21. Rats in the massed:remote group (n = 10) were massed-trained from P18-20 and tested at P41. Rats in the spaced:recent group (n = 9) were spaced-trained from P18-20 and tested at P21. Rats in the spaced:remote group (n = 9) were spaced-trained from P18-20 and tested at P41.

To determine more accurately when the emergence of remote spatial memory capabilities in preadolescent rats resembles those of adults, rats were sorted into 3 different age groups when they underwent water maze training. All rats were then tested on the water maze 3 weeks following their last training day. Rats in the first group (n = 8) were trained on the water maze from P20-22 and tested on P43 (P20-22:remote). Rats in the second group (n = 8) were trained on the water maze from P22-24 then tested on P45 (P22-24:remote). Rats in the third group (n = 9) were trained on the water maze from P24-26 and tested on P47 (P24-26:remote).

2.6. Immunohistochemistry

One hour after the end of the probe test, brains were removed from the skull and each condition immersion-fixed in 4% paraformaldehyde in 0.1M phosphate-buffer (PB) overnight at 4°C. This solution was replaced with 30% sucrose in 0.1M PB the following day and brains were stored at 4°C until sectioning. Brains were sectioned through the anterior cingulate cortex and the dorsal hippocampus at 60µm on a Leica CM1900 cryostat (Weztler, Germany). Sections were stored in a 0.1% sodium azide solution in 0.1M phosphate buffer (PB) at 4°C.

The ACC (P18-20: n = 6 for recent, n = 4 for remote; P50-52: n = 5 for recent, n = 5 for remote; massed: n = 4 for recent, n = 4 for remote; spaced: n = 3 for recent, n = 4 for remote;

P20-22: n = 4; P22-24: n = 6; P24-26: n = 4) and CA1 (P18-20: n = 7 for recent, n = 7 for remote; P50-52: n = 3 for recent, n = 6 for remote; massed: n = 3 for recent, n = 3 for remote; spaced: n = 4 for recent, n = 4 for remote; P20-22: n = 4; P22-24: n = 3; P24-26: n = 3) were stained for c-Fos. Sections were placed in phosphate-buffered saline with Triton X (PBS-TX) for three, 7-minute washes. They were then incubated in 0.3% hydrogen peroxide (H₂O₂) in PBS-TX for 30 minutes, followed by three, five-minute washes in PBS-TX. Sections were transferred to 1x animal free blocker (AFB; Vector) in PBS-TX for 30 minutes at room temperature. Incubation in the primary antibody (rabbit anti-c-Fos from Santa Cruz, 1:10000) occurred for 48 hours at room temperature. The tissue was washed in PBS-TX for three, 10-minute washes followed by a 2-hour incubation in the secondary antibody (anti-rabbit biotinylated from Vector Laboratories, 1:500). Tissue was washed for three, 10-minute washes in PBS-TX before being placed into an ABC solution (Vector) for one hour. The tissue was rinsed in three, 5-minute washes using PBS before being placed into a nickel-enhanced DAB solution. Sections were then mounted on glass slides, dehydrated, and cover slipped.

2.7. Quantification

For unbiased stereology, the CA1 and anterior cingulate areas were traced at 2.5X magnification. Cell counts were performed at 60X magnification using parameters of 60µm by 60µm and an optical fractionator of 20µm. Two sections from each rat were counted and the estimated total by mean measured thickness was analyzed.

2.8. Statistical analyses

Training indices for the first two investigations were analyzed using a fixed factor multivariate test (age and testing interval as the fixed factors, day as the repeated measure). Main effects were followed up with Tukey post-hoc tests. Probe test data were analyzed by comparing time in target quadrant and time in platform area (counter) against chance performance using a one-sample T test (quadrant chance = 25%; counter chance = 6.48%). Another analysis was a fixed factor univariate ANOVA (training group and testing interval as the fixed variables, time in target quadrant/counter as the dependent variables) followed up with Tukey post-hoc tests. Immunohistochemistry results were analyzed using a fixed factor univariate ANOVA (training group and testing interval as the independent variables, cell count as the dependent variables) and Tukey post-hoc. Data are graphically represented as mean $\pm$ SEM.

Training indices for the third investigation were analyzed using a fixed factor multivariate test (age as the fixed factor, day as the repeated measure) and Tukey post hoc tests. Main effects were followed up with Tukey post-hoc tests. Probe test data were analyzed by comparing time in target quadrant and time in platform area (counter) against chance performance using a one-sample T test (quadrant chance = 25%; counter chance = 6.48%). Another analysis was a one-way ANOVA (age group as the independent variable, time in target quadrant/counter as the dependent variables) followed up with Tukey post-hoc tests. Immunohistochemistry results were analyzed using a one-way ANOVA (age group as the independent variable, cell count as the dependent variables) and Tukey post-hoc. Data are graphically represented as mean $\pm$ SEM.

3. Results

3.1. Juvenile vs adult

This study aimed to investigate whether juvenile spatial memories acquired during the period of hippocampal synaptic modification persist beyond 24 hours and stabilized into the post-juvenile period and compare this to adult-like processing. Rats were trained over 3 days on the water maze task from P18-20 (juvenile) or P50-52 (adult) and tested at either a recent timepoint (24 hours after the last water maze training day) or a remote timepoint (3 weeks after the last water maze training day).

3.1.1. Average latency

Average latency data for the P18-20 and P50-52 groups are shown in Figure 1A. These data show that the average latencies for the P50 group were lower compared to the P18 group on each training day. The data also show that each group was consistently lower than the previous day though the P50-52 group showed superior performance (Fig 1A). A fixed factor ANOVA revealed main effects of day ($F_{(2, 56)} = 96.005, p < 0.001$), and age group ($F_{(1, 28)} = 239.872, p < 0.001$), but no main effect of testing interval ($F_{(1, 28)} = 0.353, p = 0.557$). The analysis also revealed a significant day by age group interaction ($F_{(2, 56)} = 10.590, p < 0.001$), but no other interactions. Tukey's post-hoc tests on the main effect of age revealed lower latencies in the P50-52 group than the P18-20 group ($p < 0.001$).

Figure 1A. Latency to reach the hidden platform averaged across eight trials of daily training for P18-20:recent, P18-20:remote, P50-52:recent and P50-52:remote groups. (***) indicates main effect of training day ($p < 0.001$). (###) indicates main effect of age group ($p < 0.001$). The average latency of both P50-52 groups was significantly lower than both P18-20 groups ($p < 0.001$), suggesting that the older rats acquired the memory more efficiently relative to their younger cohorts. By the second training day, the older rats appear to have reached asymptotic performance, while the latencies of the younger rats continue to decline into the third training day.

3.1.2. Average pathlength

Average pathlength data for the P18-20 and P50-52 groups are shown in Figure 1B. Pathlength data resemble the latency data in that the P50-52 groups showed superior performance but that the P18-20 groups showed improvement over days (Fig 1B). A fixed factor ANOVA revealed a main effect of day ($F_{(2, 56)} = 59.698, p < 0.001$), age group ($F_{(1, 28)} = 263.377, p < 0.001$), but no main effect of testing interval ($F_{(1, 28)} = 0.549, p = 0.465$). A significant day by age group interaction ($F_{(2, 56)} = 7.559, p < 0.01$), day by testing interval interaction ($F_{(2, 56)} = 4.099, p < 0.05$), and age group by testing interval interaction ($F_{(1, 28)} = 5.908, p < 0.05$). Tukey's post-hoc tests on the main effect of age revealed shorter pathlengths in the P50-52 group than the P18-20 group ($p < 0.001$).

Figure 1B. Pathlength to reach the hidden platform averaged across eight trials of daily training for P18-20:recent, P18-20:remote, P50-52:recent and P50-52:remote groups. (***) indicates main effect of training day ($p < 0.001$). (###) indicates main effect of age group ($p < 0.001$). The analyses revealed that the average pathlength of both P50-52 groups was significantly lower than both P18-20 groups ($p < 0.001$). Significant differences were observed on the first, second and third training days where the average pathlength of both P50-52 groups was significantly lower than both P18-20 groups ($p < 0.01$). These results show that the older rats acquired the spatial location of the platform more efficiently than their younger cohorts. This suggests that by P18, rats are capable of processing some spatial information and retaining that information for at least 24 hours yet have not reached adult-level processing capabilities.

3.1.3. Target quadrant

The percent of time spent within the target quadrant for the P18-20 and the P50-52 groups is shown in Figure 1C. This figure shows that the older groups tested recently or remotely spent more time in the target quadrant than the younger groups (Fig 1C). A one-sample t-test revealed that the time spent in the target quadrant for the P18-20:recent ($t_{(6)} = 1.110, p = 0.310$), P18-20:remote ($t_{(8)} = 0.170, p = 0.869$) groups were not significantly greater than chance. The time spent in the target quadrant for the P50-52:recent ($t_{(8)} = 2.656, p < 0.05$) and the P50-52:remote ($t_{(6)} = 2.746, p < 0.05$) groups was significantly greater than chance. A fixed factor ANOVA revealed a main effect of age group ($F_{(1, 28)} = 5.937, p < 0.05$), but no main effect of testing interval ($F_{(1, 28)} = 0.007, p = 0.935$), or age by testing interval interaction ($F_{(1, 28)} = 1.170, p = 0.289$). Tukey's post-hoc tests on the main effect of age revealed significantly longer time spent in the target quadrant for the P50-52 group than the P18-20 group ($p < 0.05$).

Probe Test: Target Quadrant

Figure 1C. Percent time spent in the quadrant associated with the former platform location during the probe test for P18-20:recent, P18-P20:remote, P50-52:recent and P50-52:remote groups. (!) indicates significant difference from chance and from the P18-20 group ($p < 0.05$).

3.1.4. Target counter

Figure 1D shows that both adult groups spent more time in the target counter relative to the juvenile groups, with the P18-20:remote group showing the least amount of time in the target counter. A one-sample t-test revealed that the P18-20:recent ($t_{(6)} = -2.625$; $p < 0.05$) and the P18-20:remote ($t_{(8)} = -5.390$; $p < 0.01$) groups performed significantly less than chance, while the P50-52:recent ($t_{(8)} = 3.011$, $p < 0.05$) and P50-52:remote group ($t_{(6)} = 2.524$, $p < 0.05$) performed significantly greater than chance. A fixed factor ANOVA revealed a main effect of age group ($F_{(1, 28)} = 27.990$, $p < 0.001$), but no main effect of testing interval ($F_{(1, 28)} = 0.041$, $p = 0.841$), or age by testing interval interaction ($F_{(1, 28)} = 2.881$, $p = 0.101$). Tukey's post-hoc tests on the main effect of age revealed significantly longer time spent in the target counter for the P50-52 group than the P18-20 group ($p < 0.001$).

Probe Test: Target Counter

Figure 1D. Percent time spent in the counter associated with the former platform location during the probe test for P18-20:recent, P18-20:remote, P50-52:recent and P50-52:remote groups. (#) indicates significant difference from chance ($p < 0.05$). (##) indicates significant difference from chance ($p < 0.01$). (***) indicates significant difference from the P18-20 group ($p < 0.001$). Despite showing signs of learning the task, rats trained from P18-20 and tested 3 weeks later were unable to retain the acquired information.

3.1.5. Immunohistochemistry

Mean c-Fos labeled cell density for the CA1 region of the hippocampus for the four groups is shown in Figure 1E. A fixed factor ANOVA revealed no main effect of age group ($F_{(1, 19)} = 3.337, p = 0.083$), or age group by testing interval interaction ($F_{(1, 19)} = 3.264, p = 0.087$), but there was a main effect of testing interval ($F_{(1, 19)} = 38.257, p < 0.001$).

Mean c-Fos labeled cell density in the ACC for the four groups is shown in Figure 1F. A fixed factor ANOVA revealed a main effect of age group ($F_{(1, 16)} = 308.706, p < 0.001$), and testing interval ($F_{(1, 16)} = 30.681, p < 0.001$), as well as a significant age group by testing interval interaction ($F_{(1, 16)} = 274.730, p < 0.001$). A Tukey post-hoc test revealed that the P18-20:recent group had significantly more activation in the ACC compared to the P18-20:remote ($p < 0.001$) group. The analysis also revealed significantly more activity in the ACC for the P50-52:recent group compared to the P18-20:remote ($p < 0.001$) group. Finally, the analysis revealed significantly more activity in the ACC for the P50-52:remote group compared to all other groups ($p < 0.001$).

Figure 1E-F. (E) Quantification and (F) representative images of c-Fos staining in the CA1 region of the hippocampus.

Figure 1G-H. (G) Quantification and (H) representative images of c-Fos staining in the ACC for the P18-20:recent, P18-20:remote, P50-52:recent and P50-52:remote groups. (!!!) indicates significant differences between the P18-20:recent and the P18-20:remote groups ($p < 0.001$). (+++) indicates significant differences between the P50-52:recent and the P18-20:remote group. (^^^) indicates significant differences between the P50-52:remote and all other groups ($p < 0.001$). The performance during the probe test parallels the level of activity observed in each brain region.

3.2. Massed vs. spaced training

Rats were sorted into 2 different training groups (massed-trained or spaced-trained) and underwent water maze training from P18-20, followed by a single-trial probe test either 24 hours or 3 weeks following their last training day. The massed-trained group underwent trials 1-8 in successive order, while the spaced-trained group received an intertrial interval from trials 1-8. This was done to identify whether spaced training would facilitate the stabilization of juvenile-acquired memories at the remote time point.

3.2.1. Average latency

Average latency data for the massed-trained and spaced-trained groups are shown in Figure 2A. These data revealed that latencies across all groups decreased over the three training days but there was no benefit of spaced training (Fig 2A). A fixed-factor ANOVA revealed main effects of day ($F_{(2, 68)} = 134.921, p < 0.001$), but no main effect of training group ($F_{(1, 34)} = 1.694, p = 0.202$) or testing interval ($F_{(1, 34)} = 0.003, p = 0.958$) and no interactions.

Figure 2A. Latency to reach the hidden platform averaged across three training days for massed:recent, massed:remote, spaced:recent, and spaced:remote groups. (***) indicates main effect of training day ($p < 0.001$). These results suggest that, while performance on the water maze improved with each passing training day, spaced trials do not increase the efficiency of spatial memory acquisition.

3.2.2. Average pathlength

Average pathlength data for massed-trained and spaced-trained groups are shown in Figure 2B. These data revealed no observable differences in pathlength between spaced and massed trials (Fig 2B). A multivariate fixed factor ANOVA revealed main effects of day ($F_{(2, 68)} = 61.967, p < 0.001$), but no main effect of training group ($F_{(1, 34)} = 1.479, p = 0.232$) or testing interval ($F_{(1, 34)} = 0.093, p = 0.762$). There was a significant day by training group interaction ($F_{(2, 68)} = 6.421, p < 0.01$), but no other interactions. Tukey's post-hoc on the day by training group interaction revealed shorter pathlengths in the massed-trained relative to the spaced-trained group on the first training day ($p < 0.05$), and significantly lower pathlengths in the spaced-trained relative to the massed-trained groups on the third training day ($p < 0.05$).

Figure 2B. Pathlength to reach the hidden platform averaged across three training days for massed:recent, massed:remote, spaced:recent, and spaced:remote groups. (***) indicates main effect of training day ($p < 0.001$). (!) indicates significantly lower pathlength in the massed-trained group. (^) indicates significantly lower pathlength in the spaced-trained group. These results suggest that, while performance on the water maze improved over time, increasing the inter-trial interval during training did not increase spatial acquisition rates.

3.2.3. Target quadrant

The percent time spent in the target quadrant for the massed- and spaced-trained groups is shown in Figure 2C. A one-sample t-test revealed that the percent time spent in the target quadrant for the massed:recent ($t_{(9)} = -0.669, p = 0.521$), massed:remote ($t_{(9)} = -2.015, p = 0.075$), spaced:recent ($t_{(8)} = 0.387, p = 0.709$), and the spaced:remote ($t_{(8)} = 0.116, p = 0.910$) was not greater than chance. A fixed factor ANOVA revealed no main effect of training group ($F_{(1, 34)} = 1.644, p = 0.208$), testing interval ($F_{(1, 34)} = 0.305, p = 0.584$), or training group by testing interval interaction ($F_{(1, 34)} = 0.064, p = 0.801$).

Probe Test: Target Quadrant

Figure 2C. Percent time spent in the quadrant associated with the former platform location during the probe test for massed:recent, massed:remote, spaced:recent, and spaced:remote groups.

3.2.4. Target counter

The percent time spent in the target counter for the massed- and spaced-trained groups is shown in Figure 2D. A one-sample t-test revealed that the massed:recent ($t_{(9)} = -0.590, p = 0.570$), spaced:recent ($t_{(8)} = 1.315, p = 0.225$), and the spaced:remote ($t_{(8)} = 0.236, p = 0.819$) spent no significant difference from chance time in the target quadrant, while the massed:remote group spent significantly less than chance time in the target counter ($t_{(9)} = -4.346, p < 0.01$). A two-way ANOVA revealed no main effect of training group ($F_{(1, 34)} = 3.813, p = 0.059$), testing interval ($F_{(1, 34)} = 1.274, p = 0.267$), or training group by testing interval interaction ($F_{(1, 34)} = 0.003, p = 0.956$).

Probe Test: Target Counter

Figure 2D. Percent time spent in the counter associated with the former platform location during the probe test for massed:recent, massed:remote, spaced:recent, and spaced:remote groups. (**) indicates significant difference from chance ($p < 0.05$).

3.2.5. Immunohistochemistry

Mean c-Fos labelled cell density for the CA1 region of the hippocampus for the massed- and spaced-trained groups is shown in Figure 2E. A fixed factor ANOVA revealed a main effect of training group ($F_{(1, 10)} = 6.582, p < 0.05$) and testing interval ($F_{(1, 10)} = 7.228, p < 0.05$), but no significant training group by interval interaction ($F_{(1, 10)} = 0.623, p = 0.448$).

Mean cell density for the ACC of massed- and spaced-trained groups is shown in Figure 2F. A fixed factor ANOVA revealed no main effect of training group ($F_{(1, 11)} = 4.505, p = 0.057$), but a main effect of testing interval ($F_{(1, 11)} = 14.979, p < 0.01$) and a significant training group by interval interaction ($F_{(1, 11)} = 11.969, p < 0.01$). Tukey's post hoc revealed significantly more staining in the spaced:remote group compared to all other groups ($p < 0.01$).

E.

F.

Figure 2E-F. (E) Quantification and (F) representative images of c-Fos staining in the CA1 region of the hippocampus for the massed:recent, massed:remote, spaced:recent, and spaced:remote groups. (**) indicates significant differences between the spaced:remote group and all other groups ($p < 0.01$). These data, combined with the probe test data, suggest that spaced training may have stabilized the memory trace into remote stores more effectively than massed training, as evidenced by the increased ACC labeling during remote testing.

G.

H.

Figure 2G-H. (G) Quantification and (H) representative images of c-Fos staining in the ACC for the massed:recent, massed:remote, spaced:recent, and spaced:remote groups. (**) indicates significant differences between the spaced:remote group and all other groups ($p < 0.01$).

3.3. P20-22:remote vs. P22-24:remote vs. P24-26:remote

Rats were sorted into 3 different juvenile age groups (P20-22:remote; P22-24:remote; P24-26:remote) and underwent water maze training, followed by a single-trial probe test 3 weeks following their last training day. This was done to determine at what point in juvenile development the ability to stabilize juvenile-acquired memories persists to remote storage similar to adults.

3.3.1. Average latency

Average latency data for the P20-22:remote, P22-24:remote and P24-26:remote groups are shown in Figure 3A. These data show that latencies across all groups decreased over the three training days but that latencies on Day 3 for the P20-22:remote group were elevated (Fig 3A). A fixed-factor ANOVA revealed main effects of day ($F_{(2, 44)} = 84.962, p < 0.001$), but no main effect of age group ($F_{(2, 22)} = 2.540, p = 0.102$). The analysis also revealed a significant day by age group interaction ($F_{(4, 44)} = 3.845, p < 0.01$). Tukey's post-hoc test revealed significant differences on the third training day where the average latency of both the P22-24:remote and the P24-26:remote groups were significantly lower than the P20-22:remote group ($p < 0.001$).

Figure 3A. Latency to reach the hidden platform averaged across eight trials of daily training for the P20-22:remote, P22-24:remote, and P24-26:remote groups. (***) indicates main effect of training day ($p < 0.001$). (###) indicates significant difference on the third training day, where the latency of the P22-24:remote and P24-26:remote groups were significantly lower than that of the P20-22:remote group ($p < 0.001$). While each age group appeared to acquire spatial information over each training day, it appeared as though the P22-24:remote and P24-26:remote groups were more developed in terms of their spatial processing capabilities, as evidenced by the asymptotic performance by the third training day.

3.3.2. Average pathlength

Average pathlength data for the P20-22:remote, P22-24:remote and P24-26:remote groups are shown in Figure 3B. These data reflect what was observed with the latencies (Fig 3A and 3B). A fixed factor ANOVA revealed a main effect of day ($F_{(2, 44)} = 80.102, p < 0.001$) but no main effect of age group ($F_{(2, 22)} = 1.958, p = 0.165$). A significant day by age group interaction ($F_{(4, 44)} = 6.147, p < 0.01$) was observed. Tukey's post-hoc tests revealed significant differences on the first training day, where the average pathlength of both the P22-24:remote and P24-26:remote groups was significantly higher than the P20-22:remote group ($p < 0.05$), and on the third training day where the average pathlength of both the P22-24:remote and P24-26:remote groups was significantly lower than the P20-22:remote group ($p < 0.01$).

Figure 3B. Pathlength to reach the hidden platform averaged across eight trials of daily training for the P20-22:remote, P22-24:remote, and P24-26:remote groups. (***) indicates main effect of training day ($p < 0.001$). (#) indicates significant difference on the first training day, where the pathlength of the P22-24:remote and P24-26:remote groups were significantly higher than that of the P20-22:remote group ($p < 0.05$). (!!!) indicates significant difference on the third training day, where the pathlength of the P22-24:remote and P24-26:remote groups were significantly higher than that of the P20-22:remote group ($p < 0.05$). While groups at each age acquired spatial information, the P22-24:remote and P24-26:remote groups were better than the P20-22:remote group as evidenced by the asymptotic performance by the third training day. This suggests that at some point between P22 and P26, rats have attained adult-like spatial processing capabilities.

3.3.3. Target quadrant

The percent of time spent in the target quadrant for the P20-22:remote, P22-24:remote, and P24-26:remote groups is shown in Figure 3C. The performance of all groups was equivalent to chance (Fig 3C) as confirmed by one-sample t-tests that revealed no significant difference between the time spent in the target quadrant and chance for the P20-22:remote ($t_{(7)} = -0.049, p = 0.963$), the P22-24:remote ($t_{(7)} = -0.286, p = 0.783$), or the P24-26:remote ($t_{(8)} = -1.314, p = 0.225$) groups. A one-way ANOVA also revealed no significant difference in time spent in the target quadrant between the age groups ($F_{(2, 22)} = 0.275, p = 0.762$).

Probe Test: Target Quadrant

Figure 3C. Percent time spent in the quadrant associated with the former platform location during the probe test for the P20-22:remote, P22-24:remote, and P24-26:remote groups. While the training provided evidence that each age group acquired spatial information, their remote probe test performance indicates that the acquired information was not transferred into remote stores. As such, it is evident that the ability to consolidate memory traces into remote stores has not fully reached adult-like levels until after P26.

3.3.4. Target counter

The percent of time spent within the target counter for the P20-22:remote, P22-24:remote, and P24-26:remote groups is shown in Figure 3D. A one-sample t-test revealed that the P20-22 ($t_{(7)} = -2.408, p < 0.05$) group spent significantly less than chance time in the target counter. There was no significant difference between the time spent in the target counter and chance for the P22-24:remote group ($t_{(7)} = -0.361, p = 0.729$) or the P24-26:remote group ($t_{(8)} = -0.416, p = 0.688$). A one-way ANOVA revealed no main effect of age group ($F_{(2, 22)} = 0.189, p = 0.829$).

Probe Test: Target Counter

Figure 3D. Percent time spent in the counter associated with the former platform location during the probe test for the P20-22:remote, P22-24:remote, and P24-26:remote groups. (*) indicates significant difference in time spent in the target counter between the P20-22:remote and P24-26:remote groups ($p < 0.05$). The P24-26:remote group outperformed the two other groups, suggesting that some of the information was processed into remote stores. As such, it is evident that while the ability to consolidate memory traces into remote memory has not fully reached adult-like levels by P26, it likely develops shortly after.

3.3.5. Immunohistochemistry

Mean c-Fos-labeled cell density for the CA1 region of the hippocampus is shown in Figure 3E. A one-way ANOVA revealed no significant difference in CA1 activity between the three conditions ($F_{(2, 9)} = 1.908, p = 0.218$).

Mean c-Fos-labeled cell density for the ACC is shown in Figure 3F. A one-way ANOVA revealed no significant difference in ACC activity between the three conditions ($F_{(2, 13)} = 1.966, p = 0.186$).

E.

F.

Figure 3E-F. (E) Quantification and (F) representative images of c-Fos staining in the CA1 region of the hippocampus for the P20-22:remote, P22-24:remote, and P24-26:remote groups.

G.

H.

Figure 3G-H. (G) Quantification and (H) representative images of c-Fos staining in the ACC for the P20-22:remote, P22-24:remote, and P24-26:remote groups. Combined with their probe test performance, these results suggest that the ability to store remote memories is not apparent by P26. Variations in activity between the hippocampus and ACC throughout the three age groups shows a trend of increased ACC activation and concurrent decrease in hippocampal activation during the remote test.

4. Discussion

One main purpose of this study was to investigate whether juvenile spatial memories acquired during a period of hippocampal development persisted beyond 24 hours and stabilized as a remote memory. A second purpose was to examine the neural activity profiles, using c-Fos, in the hippocampus and ACC to determine whether remote spatial memories are processed similarly in juveniles and adults.

Water maze training results revealed that the juvenile- (P18, P20, P22 and P24) and adult (P50)-trained groups showed evidence of learning the water maze task, as indicated by a decrease in both latency and pathlength across days. The P22 and P24 groups showed similar acquisition profiles as the P50 group suggesting adult-like spatial learning behavior. The P18 group showed significantly worse learning than the P50 group and spaced training trials did not improve their spatial learning ability. The P20 group showed close to adult-like spatial acquisition but deficits were noted on the third training day. These results indicate a developmentally linked improvement in spatial learning that reaches adult-like levels around P20 to P22.

For rats, P15 to P24 is reportedly a period during which the hippocampus undergoes extensive remodeling, denoted by an increase in neurogenesis and morphological alterations that coincide with the emergence of spatial processing (Altman and Das, 1965; Bayer, 1980; Holahan et al., 2006; Curlik et al., 2014; Holahan et al., 2019). Thus, the emergence of spatial processing may reflect a dependency on hippocampal maturation and the changes in neuronal numbers, dendritic arborization, and synaptic plasticity (Altman and Bayer, 1975; Harris and Teyler, 1984; Rahimi and Claiborne, 2007; Akers et al., 2012). Results reported in this investigation

corroborate the notion that a fully mature hippocampus contributes to the strengthening and initial processing of spatial information.

During the probe tests, the P50 group showed intact recent (24 hours) and remote (3 week) spatial memory as evidenced by above chance time spent in the target quadrant and platform location. Neither the massed- nor spaced-trained P18 groups did not show a reliable recent or remote spatial memory for the target quadrant or platform location. Likewise, the P20, P24 and P26 groups did not show evidence of a remote spatial memory for the target quadrant nor platform location. Despite evidence suggesting that they had acquired the spatial information necessary to complete the task, given their daily improvements in performance during training, the probe test revealed that the information was poorly retained when tested at a remote timepoint. These results suggest that remote spatial memory abilities emerge after P26, and that spaced-training did not facilitate the formation of remote memories.

Taking all these results together, we can surmise a general developmental emergence of spatial memory processing whereby from about P20 to P22, rats are able to process and utilize spatial information in the short term (Holahan et al., 2019). However, the ability to recall this information at remote time points (3 weeks) likely emerges after P26. Other studies investigating remote memory processing capabilities using the retention and extinction of contextual fear memories support this developmental trajectory. Campbell and Campbell (1962) performed a developmental study in rats on the retention and extinction of learned fear as a function of age. They conditioned five different age groups of rats (P18, 23, 38, 54, and 100 days) then tested their avoidance either 0 (immediately following conditioning), 7, 21, or 42 days after conditioning. When all of the age groups were tested at the shorter interval, they moved from the “shock” side to the “safe” side of the cage within the first 6 minutes of the test and stayed there

for the remainder of the test indicating an intact memory. Increasing the testing interval to 7 days resulted in only a moderate avoidance of the fear side in the P18 and P23 groups compared to the P38 and older groups. Increasing the test interval to 42 days resulted in an absence of memory for the shock side in the P18 and P23 groups, while the older groups showed an intact memory that was similar to that displayed at the immediate test time (Campbell and Campbell, 1962). In another study, rats trained at P15 showed little to no freezing at delays longer than 1 day, while rats trained at P30 and P60 showed high levels of freezing at delays of 28 days (Akers et al., 2012). Guskjolen et al. (2017) reported similar findings after assessing spatial retention in mice that ranged in age from 15 to 150 days-old at the beginning of water maze training. While all age groups exhibited intact spatial memories when tested one day after training, only the mice that were trained at 20 days or older were capable of retaining the acquired memory when tested one month later.

Systems consolidation hypotheses posit that the initial acquisition and retention of a recently-acquired memory is largely dependent on the hippocampus (Frankland and Bontempi, 2005). As time passes, the memory is transferred to cortical regions via hippocampal “teaching”, i.e. integrating the information from hippocampus to the cortex and the cortico-cortical connections are strengthened through recall, eventually leading the memory to become hippocampus-independent (Squire and Alvarez, 1995; Nadel and Moscovitch, 1997; Dudai, 2004). As such, during remote memory tests, the ACC has been a region of interest showing elevated levels of activity (Weible et al., 2012; Einarsson et al., 2015; Pezze et al., 2017). Relating our behavioral data during the probe test with the c-Fos staining revealed that in the P50 group, the hippocampal-cortical connections had been established allowing for spatial information to be consolidated into and recalled from the remote stores within the ACC,

resulting in increased ACC activity and improved performance when tested remotely. The P18 group showed a lack of remote memory recall that coincided with a lack of ACC activation. This suggests that while the P18 group showed spatial learning, there was a lack of remote memory formation indicating the possibility that the ACC had not fully developed at this developmental time period.

With this in mind, we sought to determine if changing the training method would facilitate remote memory storage during this period of hippocampal development. Whether the rats were massed-trained or spaced-trained did not impact the acquisition of the spatial information. These results parallel some findings in the literature (Spreng et al. (2002) and Kraemer and Randall, (1995)), but not all. Some reports have shown an advantageous effect of spaced-training such that spaced-trained groups show either lower latencies or reduced errors during training relative to their massed-trained counterparts (Commins et al., 2003; Bello-Medina et al., 2013; Wingard et al., 2015). An explanation for the discrepancy between the current findings and these reports may lie in the parameters used to define massed and spaced training. Massed-training in the other reports involved sequential training within the same day, whereas the spaced-training protocol distributed the training trials over multiple days. As Wingard et al. (2015) note, this may potentially create a bias towards spaced-training, as it would allow for remote consolidation processes during sleep which would not be present in massed-trained animals. Their design, similar to that of the current study, avoids this potential confound by providing an equal amount of training days between the massed- and space-training manipulations (Wingard et al., 2015).

The spaced-trained P18 group performed at chance on both testing indices, similarly to their massed-trained counterparts, when tested both recently and remotely, even though rats at

this age are reportedly capable of maintaining a viable memory for 24 hours (Campbell and Campbell, 1962; Rudy et al., 1987; Akers et al., 2012). These results conflict with the advantageous effects of spaced-training that have been reported (Kraemer and Randall, 1995; Spreng et al., 2002; Commins et al., 2003; Bello-Medina et al., 2013; Wingard et al., 2015). One likely possibility for this discrepancy may be that the intertrial interval given to the spaced-trained group was not long enough to merit any beneficial effect from this type of training. Furthermore, the state of hippocampal development at this time may be such that it requires a longer interval than that which was provided in order to facilitate the processing of spatial information, along with ensuring the retention of that information. Although the spaced-trained group did not outperform the massed-trained group, their performance was still marginally better, suggesting that spaced-training could provide some benefit to memory processing during this time of hippocampal remodeling, i.e. facilitating the emergence of adult-like memory processing, but that the intertrial interval necessary to facilitate such processes needs to be longer than the one provided in order to do so.

We also wanted to identify a time frame when juvenile-like remote retrieval more closely matched the adult-like remote processing. To accomplish this, rats were trained on the water maze during three developmental timepoints (P20-22; P22-24; P24-26). All groups were tested 3 weeks (remote test) following their last training day. Results revealed that by Day 3, the P22 and P24 groups showed adult-like spatial processing. The P20-22 group showed slightly higher latencies and longer pathlengths on Day 3 compared to the P22 and P24 groups. This suggests that sometime between P22 and P26, the efficiency to acquire spatial information increases such that it resembles that seen in adults.

While performance on spatial tasks improves incrementally during the P18 – P24 postnatal period of hippocampal remodeling, a more drastic improvement can be seen after P22, well into the remodeling process (around P24 and P26) (Rudy et al., 1987; Keeley et al., 2010; Wartman et al., 2012). Thus, it is possible that the progression of hippocampal connectivity and functionality observed by P24 may mediate the transition from juvenile- to adult-like spatial memory acquisition (Holahan et al., 2006; Comba et al., 2015).

The time spent in the target quadrant for the P20, P22 and P24 groups remained at chance at the remote test suggesting their performance was not adult-like. The time spent in the region associated with the platform (counter) was also below chance for the P20 and P22 groups though showed an increase in the P24 group. However, their performance still did not reach adultlike levels suggesting the remote memory retrieval capacity had not yet fully developed in these groups.

Compared to the P50 group, the c-Fos staining patterns for the P20, P24 and P26 groups during the remote memory test revealed fewer c-Fos positive cells in the CA1 region and a higher number of c-Fos positive cells in the ACC. It is not exactly clear why this pattern emerged, but it may be the case that the P20-P26 period reflects a sensitive period for the ACC to develop. From approximately P24 to P30, there is a reduction in the colocalization of astrocytes with dendritic markers in the infralimbic (medial prefrontal) cortex (Testen et al., 2019). Markers of pre- and post-synaptic maturation show prolonged development in the prefrontal cortical areas (including the ACC) that reach adult like levels around P36 for the pre-synaptic index (starting around P29) and P30 for the post-synaptic index (starting around P22; Pinto et al. (2013)). Levels of immediate early genes, c-Fos, zif268 and Arc are elevated in P17 and P24 rats compared to adults (P80; Jia et al. (2018)). An RNA-sequencing (RNA-seq) study

of the rat medial prefrontal cortex (mPFC) at five developmental time points revealed a change in gene expression patterns from regulation of network formation to maintenance around P21 (Kroeze et al., 2018). The authors suggest that this change in gene expression patterns may contribute to the formation of a mature PFC that underlies the performance of cognitive and emotional tasks less susceptible to the environmental factors during development (Kroeze et al., 2018). These studies suggest that the timepoints we used in the present study (P20, P22 and P24 for training and P43, P45, P47 for testing) represent a time period when significant changes occur in the development of frontal-cortical circuits including the ACC (Drzewiecki et al., 2016). Therefore, the higher activity levels as indicated by c-Fos labeling seen in the present work may be indicative of a continually developing ACC that overshadows any labeling that may be specific to the remote memory task. The P50 group showed fewer c-Fos-positive cells than these groups but in the case of the P50 group, elevated labeling was specific to the remote memory test. Interestingly, spaced-training, while associated with intact recent and remote memory, resulted in elevated c-Fos positive cells that was also specific to the remote memory test. This suggests that possibility that spaced-training may facilitate the developmental switch in the ACC from regulation to maintenance. These hypotheses will require further testing to validate.

5. Conclusion

The current work investigated whether juvenile spatial memories acquired during a period of hippocampal synaptic modification persist beyond 24 hours and stabilized into the post-adolescent period as a remote memory. By P18, the state of hippocampal development appears to underline the initial emergence of spatial information processing with rapid improvements until P24 when spatial information processing matches that of adults. During this same timeframe, the ability to store that spatial information into a remote memory is not apparent, likely due to underdeveloped hippocampal-cortical (ACC) connections and a reduced processing capacity of cortical networks. This ability begins to emerge after P26 when the hippocampus is developed and hippocampal-cortical connections are formed, allowing for the persistence of a memory trace into the remote time frame. The spaced-training provided could not remedy the remote processing impairment observed during the early stages of hippocampal development. This effect is likely due to the necessity of a longer intertrial interval than the one provided at this stage of hippocampal development in order to facilitate the emergence of adult-like memory processing earlier in development.

References

- Akers KG, Arruda-Carvalho M, Josselyn SA, Frankland PW (2012) Ontogeny of contextual fear memory formation, specificity, and persistence in mice. *Learn Mem* 19:598–604.
- Altman J, Bayer S (1975) Postnatal Development of the Hippocampal Dentate Gyrus Under Normal and Experimental Conditions. In: *The Hippocampus*, pp 95–122. Boston, MA: Springer US.
- Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124:319–335.
- Amaral DG, Dent JA (1981) Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions. *J Comp Neurol* 195:51–86.
- Bayer SA (1980) Development of the hippocampal region in the rat I. Neurogenesis examined with 3H-thymidine autoradiography. *J Comp Neurol* 190:87–114.
- Bello-Medina PC, Sánchez-Carrasco L, González-Ornelas NR, Jeffery KJ, Ramírez-Amaya V (2013) Differential effects of spaced vs. massed training in long-term object-identity and object-location recognition memory. *Behav Brain Res* 250:102–113.
- Bercovitz KE, Bell MC, Simone PM, Wiseheart M (2017) The spacing effect in older and younger adults: does context matter? *Aging, Neuropsychol Cogn* 24:703–716.
- Bontempi B, Laurent-Demir C, Destrade C, Jaffard R (1999) Time-dependent reorganization of brain circuitry underlying long-term memory storage. *Nature* 400:671–675.
- Campbell BA, Campbell EH (1962) Retention and extinction of learned fear in infant and adult rats. *J Comp Physiol Psychol* 55:1–8.

- Comba R, Gervais N, Mumby D, Holahan M (2015) Emergence of spatial behavioral function and associated mossy fiber connectivity and c-Fos labeling patterns in the hippocampus of rats. *F1000Research* 4.
- Commins S, Cunningham L, Harvey D, Walsh D (2003) Massed but not spaced training impairs spatial memory. *Behav Brain Res* 139:215–223.
- Curlik DM, DiFeo G, Shors TJ (2014) Preparing for adulthood: Thousands upon thousands of new cells are born in the hippocampus during puberty, and most survive with effortful learning. *Front Neurosci* 8.
- Drzewiecki CM, Willing J, Juraska JM (2016) Synaptic number changes in the medial prefrontal cortex across adolescence in male and female rats: A role for pubertal onset. *Synapse* 70:361–368.
- Dudai Y (2004) The Neurobiology of Consolidations, Or, How Stable is the Engram? *Annu Rev Psychol* 55:51–86.
- Einarsson E, Pors J, Nader K (2015) Systems reconsolidation reveals a selective role for the anterior cingulate cortex in generalized contextual fear memory expression. *Neuropsychopharmacology* 40:480–487.
- Frankland PW, Bontempi B (2005) The organization of recent and remote memories. *Nat Rev Neurosci* 6:119–130.
- Frankland PW, Bontempi B (2006) Fast track to the medial prefrontal cortex. *Proc Natl Acad Sci U S A* 103:509–510.
- Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ (2004) The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory. *Science* (80-) 304:881–883.

- Gaarskjaer FB (1985) The development of the dentate area and the hippocampal mossy fiber projection of the rat. *J Comp Neurol* 241:154–170.
- Gaarskjaer FB (1986) The organization and development of the hippocampal mossy fiber system. *Brain Res* 396:335–357.
- Guskjolen A, Josselyn SA, Frankland PW (2017) Age-dependent changes in spatial memory retention and flexibility in mice. *Neurobiol Learn Mem* 143:59–66.
- Harris KM, Teyler TJ (1984) Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. *J Physiol* 346:27–48.
- Holahan MR, Honegger KS, Routtenberg A (2007) Expansion and retraction of hippocampal mossy fibers during postweaning development: Strain-specific effects of NMDA receptor blockade. *Hippocampus* 17:58–67.
- Holahan MR, Rekart JL, Sandoval J, Routtenberg A (2006) Spatial learning induces presynaptic structural remodeling in the hippocampal mossy fiber system of two rat strains. *Hippocampus* 16:560–570.
- Holahan MR, Tzakis N, Oliveira FA (2019) Developmental Aspects of Glucose and Calcium Availability on the Persistence of Memory Function Over the Lifespan. *Front Aging Neurosci* 11.
- Jia M, Travaglia A, Pollonini G, Fedele G, Alberini CM (2018) Developmental changes in plasticity, synaptic, glia, and connectivity protein levels in rat medial prefrontal cortex. *Learn Mem* 25:533–543.
- Keeley RJ, Wartman BC, Häusler AN, Holahan MR (2010) Effect of juvenile pretraining on adolescent structural hippocampal attributes as a substrate for enhanced spatial performance. *Learn Mem* 17:344–354.

- Kraemer PJ, Randall CK (1995) Spatial learning in preweanling rats trained in a Morris water maze. *Psychobiology* 23:144–152.
- Kroeze Y, Oti M, Van Beusekom E, Cooijmans RHM, Van Bokhoven H, Kolk SM, Homberg JR, Zhou H (2018) Transcriptome analysis identifies multifaceted regulatory mechanisms dictating a genetic switch from neuronal network establishment to maintenance during postnatal prefrontal cortex development. *Cereb Cortex* 28:833–851.
- Lopez J, Herbeaux K, Cosquer B, Engeln M, Muller C, Lazarus C, Kelche C, Bontempi B, Cassel JC, de Vasconcelos AP (2012) Context-dependent modulation of hippocampal and cortical recruitment during remote spatial memory retrieval. *Hippocampus* 22:827–841.
- Maviel T, Durkin TP, Menzaghi F, Bontempi B (2004) Sites of neocortical reorganization critical for remote spatial memory. *Science* (80-) 305:96–99.
- Nadel L, Moscovitch M (1997) Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol* 7:217–227.
- Pezze MA, Marshall HJ, Fone KC, Cassaday HJ (2017) Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. *Learn Mem* 24:310–317.
- Pinto JGA, Jones DG, Murphy KM (2013) Comparing development of synaptic proteins in rat visual, somatosensory, and frontal cortex. *Front Neural Circuits* 7.
- Rahimi O, Claiborne BJ (2007) Morphological development and maturation of granule neuron dendrites in the rat dentate gyrus. *Prog Brain Res* 163:167–181.
- Rudy JW, Stadler-Morris S, Albert P (1987) Ontogeny of Spatial Navigation Behaviors in the Rat: Dissociation of “Proximal”-and “Distal”-Cue-Based Behaviors. *Behav Neurosci* 101:62–73.

- Spreng M, Rossier J, Schenk F (2002) Spaced training facilitates long-term retention of place navigation in adult but not in adolescent rats. *Behav Brain Res* 128:103–108.
- Squire LR, Alvarez P (1995) Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol* 5:169–177.
- Teixeira CM, Pomedli SR, Maei HR, Kee N, Frankland PW (2006) Involvement of the anterior cingulate cortex in the expression of remote spatial memory. *J Neurosci* 26:7555–7564.
- Testen A, Ali M, Sexton HG, Hodges S, Dubester K, Reissner KJ, Swartzwelder HS, Risher ML (2019) Region-Specific Differences in Morphometric Features and Synaptic Colocalization of Astrocytes During Development. *Neuroscience* 400:98–109.
- Vlach HA, Bredemann CA, Kraft C (2019) To mass or space? Young children do not possess adults' incorrect biases about spaced learning. *J Exp Child Psychol* 183:115–133.
- Wartman BC, Gabel J, Holahan MR (2014) Inactivation of the anterior cingulate reveals enhanced reliance on cortical networks for remote spatial memory retrieval after sequential memory processing. *PLoS One* 9.
- Wartman BC, Gervais NJ, Smith C, Comba R, Mumby DG, Holahan MR (2012) Enhanced adolescent learning and hippocampal axonal projections following preadolescent spatial exposure to a water or dry maze. *Brain Res* 1475:37–48.
- Wartman BC, Holahan MR (2013) The use of sequential hippocampal-dependent and -non-dependent tasks to study the activation profile of the anterior cingulate cortex during recent and remote memory tests. *Neurobiol Learn Mem* 106:334–342.
- Wartman BC, Holahan MR (2014) The impact of multiple memory formation on dendritic complexity in the hippocampus and anterior cingulate cortex assessed at recent and remote time points. *Front Behav Neurosci* 8.

- Weible AP, Rowland DC, Monaghan CK, Wolfgang NT, Kentros CG (2012) Neural correlates of long-term object memory in the mouse anterior cingulate cortex. *J Neurosci* 32:5598–5608.
- Wiltgen BJ, Brown RAM, Talton LE, Silva AJ (2004) New circuits for old memories: The role of the neocortex in consolidation. *Neuron* 44:101–108.
- Wingard JC, Goodman J, Leong KC, Packard MG (2015) Differential effects of massed and spaced training on place and response learning: A memory systems perspective. *Behav Processes* 118:85–89.
- Zhao C, Teng EM, Summers RG, Ming GL, Gage FH (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci* 26:3–11.

Chapter 3

Investigation of GluR1 and GluR2 AMPA receptor subtype distribution in the hippocampus and anterior cingulate cortex of Long Evans rats during development

Nikolaos Tzakis, Matthew R. Holahan
Department of Neuroscience

Manuscript submitted to: IBRO Reports

Abstract

Understanding the emergence of cognitive functions like spatial learning requires insight about the development of cellular underpinnings that mediate the transition from juvenile-like to adult-like spatial processing capabilities. In adults, the hippocampus and anterior cingulate cortex (ACC) interact for the remote storage of memories. The emergence of this function during development has not been established. A link between changes in the duration of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA)-mediated synaptic responses in the hippocampus and the emergence of spatial navigation has been shown. AMPA are made up of 4 subunits, of which GluR1 and GluR2 have been shown to play the most prominent role in cognitive processes. In this study, we sought to determine whether levels of these two subunits changed during the course of late pre-adolescent development in the hippocampus and ACC. To investigate the developmental changes in AMPA subunits, Western blotting and immunohistochemistry were performed on the ACC and hippocampus during development (P18-30) and compared to adult rats (P50) to determine the quantitative difference in GluR1 and GluR2 levels. Within the hippocampus, protein levels of GluR1 and GluR2 peaked around P26-30 whereby localized staining in the dentate gyrus reflected this pattern. GluR1 and GluR2 levels within the ACC showed little variation during this developmental period. These results indicate that while changes in AMPA subunits may contribute to the emergence of spatial processing capabilities that underlie the shift from juvenile- to adult-like capabilities, they might not mediate functional coupling between the hippocampus and ACC for remote memory storage.

1. Introduction

Hebbian theory postulates that coincident presynaptic and postsynaptic excitation leads to an increase in synaptic efficacy which results in synaptic plasticity leading to the encoding and storage of information within neurons (Fox and Stryker, 2017). Long-term potentiation (LTP) refers to a long-lasting strengthening of the relationship between pre- and post-synaptic elements following repeated stimulation that is thought to be related to learning and remote memory (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; Malinow, 2003). LTP is maintained by persistent modifications of the glutamatergic receptors, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), including an increase in their binding affinity, number of binding sites, or a change in their ion channel kinetics (Maren et al., 1993). Perforant-path LTP induction results in an increase in hippocampal AMPAR binding sites suggesting that AMPAR binding properties may regulate LTP-induced enhanced synaptic transmission observed at hippocampal synapses (Maren et al., 1993).

AMPA receptors are concentrated at synapses where they mediate fast excitatory post-synaptic currents. They are composed of a combination of four subunits, GluR1-4, with each combination of subunits dictating the distinct functional properties of the receptor (Hollmann and Heinemann, 1994; Schoepfer et al., 1994; Dingledine et al., 1999; Murphy et al., 2012; Henley and Wilkinson, 2016). The majority (~80%) of synaptic AMPAR in CA1 hippocampal neurons are composed of GluR1 and GluR2 heteromers, while subunits containing GluR3 occur at much lower rate (~10%). GluR4 is developmentally regulated, with expression peaking during early postnatal development and decreasing thereafter, such that glutamatergic synapses in principal neurons in adult brains sparsely express the GluR4 subunit (Henley and Wilkinson, 2016). In fact, GluR4-containing AMPAR delivered into synapses following spontaneous neural activity

are reportedly exchanged with non-synaptic GluR2-containing AMPAr (Zhu et al., 2000). The remaining GluR4-containing AMPAr play a role in mediating transmission in interneurons, especially parvalbumin-containing inhibitory interneurons (Henley and Wilkinson, 2016).

The GluR1 subunit has been extensively studied for its role in spatial memory processing. Several phosphorylation sites, particularly serine 831 (S831) and serine 845 (S845), have been shown to be crucial for LTP and activity-dependent GluR1 trafficking (Barria et al., 1997; Lee et al., 2000, 2010; Esteban et al., 2003). Genetically modifying these phosphorylation sites results in reduced LTP and impaired hippocampus-dependent spatial working memory (Lee et al., 2003; Sanderson et al., 2008). GluR2 confers many of the major biophysical properties of the receptor, including receptor kinetics, single-channel conductance, Ca^{+2} permeability, and receptor assembly and trafficking (Isaac et al., 2007). The majority of GluR2 mRNA exists in an edited form, changing the glutamine to arginine at position 607 and rendering any heteromeric GluR2-containing AMPAr Ca^{+2} -impermeable (Tanaka et al., 2000; Henley and Wilkinson, 2016). AMPAr present in mature neurons that lack GluR2 or contain the unedited GluR2 form are Ca^{+2} -permeable and are essential to synaptic plasticity underlying learning and memory processes (Wright and Vissel, 2012; Henley and Wilkinson, 2016). GluR2 has been shown to be involved in homeostatic synaptic scaling. This form of plasticity functions to scale synaptic transmission through a negative feedback mechanism. It resets the neuronal firing rate and has been suggested to counteract LTP to maintain network stability and prevent runaway excitation. In essence, it provides necessary negative feedback signaling while preserving the encoded information within the individual synapses (Turrigiano et al., 1998; Gainey et al., 2009; Siddoway et al., 2014).

GluR1 and GluR2 expression vary considerably throughout development. GluR2 expression is low relative to GluR1 expression around postnatal (P) days 3-5, consistent with

prior reports that attribute GluR2-lacking AMPAR to neonatal synaptic function (Pickard et al., 2000; Henley and Wilkinson, 2016). Expression of GluR1 continues to increase between P5 and P21, after which there is a shift whereby GluR1 expression levels decline and GluR3 levels increase (Pellegrini-Giampietro et al., 1992b; Pickard et al., 2000; Henley and Wilkinson, 2016). This results in a reduction in the threshold for postsynaptic LTP induction and concurrent increase in the presynaptic LTP induction threshold (Dumas, 2012; Blair et al., 2013). By P14, almost all AMPAR synapses express GluR2, suggesting a developmental “switch” from Ca^{+2} -permeable to -impermeable AMPAR during early development (Pellegrini-Giampietro et al., 1992b; Pickard et al., 2000; Henley and Wilkinson, 2016).

The developmental time course for GluR1 and GluR2 expression coincides with a phase of hippocampal remodeling and morphological modifications that occur during the late preadolescent period. Neurogenesis within the dentate gyrus reportedly peaks in density between P15 and P18 (Altman and Das, 1965; Bayer, 1980; Zhao et al., 2006; Curlik et al., 2014). Mossy fibres, which form connections between granule cells in the dentate gyrus and CA3 pyramidal cells extend from the stratum lucidum to the stratum oriens of the CA3 from P18 to P24 where they remain stable into adulthood (Amaral and Dent, 1981; Gaarskjaer, 1985, 1986; Holahan et al., 2006, 2007). This phase of hippocampal modification is hypothesized to underlie a “switch” from juvenile- to adult-like spatial memory processing (Holahan et al., 2019). What has not been investigated is the variation in GluR1 and GluR2 levels during this time of connectivity changes within the hippocampus.

The initial processing of spatial memories is reliant on hippocampal activity, but there remains some ambiguity concerning its contribution to the remote storage of those memories (Morris et al., 1982, 1986; Riedel et al., 1999; Broadbent et al., 2004). One hypothesis suggests a

gradual strengthening of cortical connections and concurrent disengagement of the hippocampus for the remote storage of information (Wiltgen et al., 2004; Frankland and Bontempi, 2005, 2006). According to this view, the involvement of the hippocampus is limited to the initial information processing, after which point the memory trace is represented by strengthened intracortical connections (Bontempi et al., 1999; Frankland et al., 2004; Maviel et al., 2004; Teixeira et al., 2006; Lopez et al., 2012). Reports have pointed to the possibility that remote memory stores are found within the anterior cingulate cortex (ACC), given the increased metabolic activity and structural changes within this region following remote memory testing (Bontempi et al., 1999; Teixeira et al., 2006; Weible et al., 2012; Wartman and Holahan, 2013, 2014; Wartman et al., 2014).

The ACC is thought to undergo a series of remodeling and structural modifications that occur slightly later in development than the hippocampus. The colocalization of astrocytes with dendritic markers are reduced between P24 to P30, while markers of pre- and post-synaptic maturation show prolonged development that reach adult like levels around P36 for the pre-synaptic index (starting around P29) and P30 for the post-synaptic index (starting around P22; Pinto et al., 2013; Testen et al., 2019). Given the contribution of GluR1 and GluR2 to memory processing and LTP, it is possible that the late synaptic remodeling of the ACC is reflected in levels of GluR1 and GluR2 expression. The coincident changes in GluR1 and GluR2 in the hippocampus and ACC may provide the necessary substrates for the transfer of recent to remote memory storage functions.

In the current investigation, Western blotting was performed on the hippocampal formation and ACC region to evaluate the variation in expression levels of GluR1 and GluR2 AMPAR subunits within the hippocampus and ACC from P18 - 30 and compare them to adult

(P50) levels. Immunohistochemical analyses were also performed on the hippocampus to localize GluR1 and GluR2 levels during development.

2. Materials and methods

2.1. Subjects

Male Long-Evans rats (LERs) were used throughout the experiment. Rats were born in the temperature and humidity-regulated animal facility at Carleton University. The day the pups were born was marked as P0. Pups were weaned on P18. Newly weaned rats were provided with solid food pellets soaked with some water and placed in a ceramic dish in the cage, along with a dish containing fortified water gel. Unsoaked food pellets were also placed in the cage. For all experiments, rats were group-housed in polycarbonate cages with a 12-hour light-dark cycle: lights on 8h00, lights off 20h00 with food and water provided *ad libitum*. Animal care was conducted in accordance with the Canadian Council on Animal Care (CCAC) guidelines and approved by the Carleton University Animal Care Committee.

2.2. Western blotting

Rats were injected with 3 ml urethane at one of seven ages (P18, P20, P22, P24, P26, P30, P50). When they were no longer responsive to a toe pinch, rats were decapitated, and brains were removed from the skull and hemisected. Tissue punches were collected from the ACC and hippocampus from one hemisphere and used to for Western blotting quantification. The other hemisphere was immersion-fixed in 4% paraformaldehyde. Hemispheres were counterbalanced for the two analyses.

Whole cell lysates from the hippocampus were homogenized in Radio Immuno Precipitation Assay (RIPA) buffer [50 mM Tris (pH 8.0), 150 mM sodium chloride, 0.1% sodium dodecyl sulphate (SDS), 0.5% sodium deoxycholate and 1% Triton X-100] mixed with 1 tablet of Complete Mini ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor (Roche Diagnostics, Laval, QC, Cat #11 836 170 001) per 10 mL of buffer then sonicated for 10 seconds in ice cold water. The lysed cells were centrifuged at 5000 RPM for 10 minutes at 4°C. The supernatant was extracted, and protein concentration was determined using bicinchoninic acid (BCA) method (Thermo Scientific). Following protein concentration determination, the supernatant was placed in 5x loading buffer (containing 5% glycerol, 5% β -mercaptoethanol, 3% SDS and 0.05% bromophenol blue) and the protein was denatured by placement in a 5-minute heating block at a temperature of 105°C. Following this step, samples were stored at -20°C.

Proteins were separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE gel (7.5%) containing the separating buffer (370 mM Tris-base (pH 8.8), 3.5 mM SDS) and the stacking buffer (124 mM Tris-base (pH 6.8), 3.5 mM SDS) were placed in running buffer (25 mM Tris-base, 190 mM glycine, 3.5 mM SDS) and samples, along with the Precision Plus Protein™ Standards Dual Color (Bio-Rad, Hercules, CA, Cat#161-0374), were loaded into the Arcylamide gel (7.5 %) for molecular weight determination at 140 volts. After electrophoresis, proteins were transferred for one hour at 4°C at 100 volts in transfer buffer solution (25 mM Tris-base, 192 mM Glycine, 20% methanol) onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Cat# 162-0177). Thereafter, membranes were dried overnight. The following day, membranes were reactivated using methanol and total protein load concentration was determined.

After a brief methanol rinse (5 seconds), membranes were incubated in REVERT total protein solution for a period of 5 minutes followed by placement into a REVERT wash solution (6.7% Glacial Acetic Acid, 30% Methanol, in water) two times 2 minutes each. Membranes were quickly rinsed with distilled water and imaged on a LI-COR Odyssey imaging system on the 700 channel for an exposure period of 2 minutes. Membranes were then rinsed immediately post imaging in tris-buffered saline [(TBS) pH 7.5 (2 X 5min.)] followed by a block with 0.5% fish gelatin (Sigma) in TBS for 60 minutes. Membranes were incubated with rabbit anti-GluR1 (1:1000; EMD Millipore, Cat# AB1504) or rabbit anti-GluR2 (1:4000; EMD Millipore, Cat# AB1768-I) overnight in 0.05% fish gelatin in TBS with 0.1% tween. Membranes were washed with 15 mL of TBS-T/membrane at room temperature three times five minutes each. Membranes were then incubated for one hour in infrared conjugate secondary (AlexaFluor 680 goat anti-rabbit; Invitrogen, Cat# AB175773) at a concentration of 1:4000 in 0.5% fish gelatin solution containing 0.2% tween and 0.01% SDS. Membranes were then washed in TBS-T (1 X 5 minutes) followed by 2 X 5 minutes washes in TBS and read on a Licor Odyssey system on the 700-channel for 6 minutes.

Total protein expression from each sample on each membrane was determined using the total protein stain method in order to provide normalization across each specific membrane. To determine protein expression, bands at the appropriate molecular weight were determined with the Odyssey software. Presented data represents the ratio of target protein signal strength to normalized total protein signal strength.

2.3. Immunohistochemistry

The hemisphere not used for Western blot analysis was immersion-fixed in 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS) overnight at 4°C. This solution was replaced with 30% sucrose in 0.1M PBS the following day and brains were stored at 4°C until sectioning. Brains were sectioned through the anterior cingulate cortex and the dorsal hippocampus at 60µm on a Leica CM1900 cryostat (Weztler, Germany). Sections were stored in a 0.1% sodium azide solution in 0.1M phosphate buffer (PB) at 4°C.

For GluR1 and GluR2 immunohistochemistry, sections were placed in phosphate-buffered saline with Triton X (PBS-TX) for three, 5-minute washes. They were then incubated in 0.3% hydrogen peroxide (H₂O₂) in PBS-TX for 30 minutes, followed by three, five-minute washes in PBS-TX. Sections were transferred to 1x animal free blocker (AFB; Vector) in PBS-TX for 30 minutes at room temperature. Incubation in the primary antibody anti-GluR1 (rabbit; 1:1000; EMD Millipore, Cat# AB1504) or anti-GluR2 (rabbit; 1:4000; EMD Millipore, Cat# AB1768-I) occurred for 24 hours at room temperature. The tissue was washed in PBS-TX for three, 10-minute washes followed by a 2-hour incubation in the secondary antibody (anti-rabbit biotinylated from Vector Laboratories, 1:500, Cat# BA-9200). Tissue was washed for three, 10-minute washes in PBS-TX before being placed into an ABC solution (Vector) for one hour. The tissue was rinsed in three, 5-minute washes using PBS before being placed into a DAB solution. Sections were then mounted on glass slides, dehydrated, and cover slipped.

2.4. Statistical analyses

For Western blotting and immunohistochemical counts, a one-way ANOVA was run with age of the rat as the independent variable and protein signal strength (Western blotting) or cell

count (immunohistochemistry) as the dependent variable. Comparisons between each age groups were conducted using Tukey test post hoc analysis when a main effect of age was present. Data are graphically represented as mean $\pm$ SEM.

3. Results

3.1. Western blotting

3.1.1. GluR1

Western blot results for the GluR1 subunit in the ACC are shown in Figure 1 for P18 (n = 3), P20 (n = 6), P22 (n = 5), P24 (n = 5), P26 (n = 5), P30 (n = 5), and P50 (n = 5). These data show that GluR1 expression in the ACC remained relatively consistent throughout the developmental ages, with a slight decrease at P22 and P24 (Fig 1). A one-way ANOVA revealed no significant difference in ACC GluR1 levels across the seven age groups ($F_{(6, 27)} = 0.977, p = 0.482$).

Western blot results for the GluR1 subunit in the HPC are shown in Figure 2 for P18 (n = 5), P20 (n = 4), P22 (n = 5), P24 (n = 5), P26 (n = 5), P30 (n = 5), and P50 (n = 5). These data show that GluR1 levels in the hippocampus sharply increased from P18 to 20, and then gradually increased and peaked at P30 and remained stable into adolescence (Fig 2). A one-way ANOVA revealed a main effect of age ($F_{(6, 27)} = 2.890, p < 0.05$). Tukey post-hoc tests revealed that hippocampal GluR1 levels at P30 were significantly higher than at P18 ($p < 0.05$).

B.

Figure 1. (A) Western blot analyses and (B) representative bands of GluR1 in the ACC of rats in the P18, 20, 22, 24, 26, 30, and 50 age groups. The consistent level of expression throughout the early developmental ages in the ACC may indicate that maturation of GluR1 occurs either earlier or later in development. Data are presented as mean $\pm$ SEM.

B.

Figure 2. (A) Western blot analyses and (B) representative bands of GluR1 in the hippocampus of rats in the P18, 20, 22, 24, 26, 30, and 50 age groups. (*) indicates significant differences between P18 and P30 groups ($p < 0.05$). This not only indicates a period of GluR1 development from P18-30, but also provides evidence that by P30, GluR1 expression has reached adult levels. Data are presented as mean $\pm$ SEM.

3.1.2. GluR2

Western blot results for the GluR2 subunit in the ACC are shown in Figure 3 for P18 (n = 3), P20 (n = 4), P22 (n = 3), P24 (n = 5), P26 (n = 5), P30 (n = 5), and P50 (n = 5). These data show that GluR2 levels in the ACC remained consistent throughout the developmental ages, with a slight increase at P50 (Fig 2A). A one-way ANOVA revealed no significant difference in ACC GluR2 across the seven age groups ($F_{(6, 23)} = 0.518, p = 0.789$).

Western blot results for the GluR2 subunit in the HPC are shown in Figure 4 for P18 (n = 3), P20 (n = 4), P22 (n = 3), P24 (n = 5), P26 (n = 5), P30 (n = 5), and P50 (n = 5).. These data show that GluR2 levels in the hippocampus gradually increased throughout development, peaking at P30 and remaining stable into adolescence. A one-way ANOVA revealed a significant main effect of age ($F_{(6, 26)} = 2.899, p < 0.05$). Tukey post-hoc tests revealed that hippocampal GluR2 levels at P30 were significantly higher than at P18 ($p < 0.05$).

B.

Figure 3. (A) Western blot analyses and (B) representative bands of GluR2 expression in the ACC of rats in the P18, 20, 22, 24, 26, 30, and 50 age groups. The consistent level of expression throughout the early developmental ages in the ACC may indicate that maturation of GluR2 occurs later in development relative to the hippocampus. Data are presented as mean $\pm$ SEM.

B.

Figure 4. (A) Western blot analyses and (B) representative bands of GluR2 expression in the hippocampus of rats in the P18, 20, 22, 24, 26, 30, and 50 age groups. (*) indicates significant differences between P18 and P30 groups ($p < 0.05$). This not only indicates a period of GluR2 development from P18-30, but also provides evidence that by P30, GluR1 expression has reached adult levels. Data are presented as mean $\pm$ SEM.

3.2. Immunohistochemistry

Dense staining within the CA1 and CA3 proved to be difficult to provide an accurate count within those regions (Fig 5). As such, counts were localized exclusively within the dentate gyrus area of the hippocampus (Fig 5) which showed punctate cellular staining.

Immunohistochemical results for the GluR1 subunit in the HPC are shown in Figure 6 for P18 (n = 4), P20 (n = 3), P22 (n = 3), P24 (n = 2), P26 (n = 4), P30 (n = 4), and P50 (n = 4). These data show that GluR1-stained cells in the dentate gyrus increased throughout development and stabilized into adulthood by P24 (Fig 6). A one-way ANOVA revealed a significant main effect of age ($F_{(6, 17)} = 8.204, p < 0.001$). Tukey post-hoc test revealed that dentate GluR1 staining at P30 was significantly higher than P18 ($p < 0.05$). P50 were significantly lower compared to all P20, 22, 24, 26, and 30 ($p < 0.05$).

Immunohistochemical results for the GluR2 subunit in the HPC are shown in Figure 7 for P18 (n = 3), P20 (n = 4), P22 (n = 6), P24 (n = 4), P26 (n = 3), P30 (n = 4), and P50 (n = 4). These data show that GluR2 staining in the dentate increased throughout development and stabilized into adulthood by P24 (Fig 6). A one-way ANOVA revealed a significant main effect of age ($F_{(6, 21)} = 12.961, p < 0.001$). Tukey post-hoc test revealed that dentate GluR2 staining at P24, 26, 30, and 50 were significantly higher than P18, and at P24, 26, and 30 compared to P20 and 22 ($p < 0.01$).

B.

Figure 5. Representative staining within the hippocampus, and CA1, CA3, and dentate gyrus (DG) subregions for (A) GluR1 and (B) GluR2. Dense staining within the CA1 and CA3 proved to be difficult to provide an accurate count within those regions. As such, counts were localized within the dentate gyrus area of the hippocampus.

B.

Figure 6. (A) Immunohistochemical analyses and (B) representative staining of GluR1 within the dentate gyrus of the hippocampus in the P18, 20, 22, 24, 26, 30, and 50 age groups. (*) indicates significant difference between P18 and P30 ($p < 0.05$). (!) indicates significant difference between the P50 group and all other age groups ($p < 0.05$). Data are presented as mean $\pm$ SEM.

B.

Figure 7. (A) Immunohistochemical analyses and (B) representative staining of GluR2 within the dentate gyrus of the hippocampus in the P18, 20, 22, 24, 26, 30, and 50 age groups. (***) indicates significant differences between the P24, 26, 30, and 50 groups and the P18, 20, and 22 groups ($p < 0.001$). (!!) indicates significant differences between the P18 and P50 groups ($p < 0.01$). Data are presented as mean $\pm$ SEM.

4. Discussion

The current investigation evaluated developmental changes in levels of GluR1 and GluR2 AMPAR subunits within the hippocampus and ACC from P18 - 30 and compared them to adult (P50) levels. This developmental period coincides with the emergence of spatial processing so we hypothesized that changes in GluR1 or GluR2 levels in the hippocampus would parallel this cognitive milestone. Shortly after the emergence of spatial processing, the ability to store memories into cortical networks may emerge. We hypothesized that levels of GluR1 and GluR2 in the anterior cingulate cortex (ACC) would parallel this cognitive ability. Consistent with the first hypothesis, GluR1 levels increased from P18 to P30 coinciding with changes in hippocampal-dependent spatial functions. GluR1 protein level changes across development were reflected in immunohistochemical localization to the dentate gyrus. GluR2 hippocampal levels showed a more linear increase from P18 to 30 while immunohistochemical staining in the dentate revealed an abrupt increase from P22 to P24. Levels of GluR1 and GluR2 in the ACC showed little variation across the developmental timepoints investigated.

Hippocampal GluR1 levels evaluated during the first four postnatal weeks dramatically increased after 3 weeks of age, with an expression pattern similar to that found in the adult appearing around P21 (Durand and Zukin, 1993; Ryo et al., 1993; Martin et al., 1998; Ibaraki et al., 1999; Hagihara et al., 2011). A gradual increase in hippocampal GluR1 expression has been reported across P0, 7, 14, and 30, with the highest levels detected at P30 (Bian et al., 2012). Sans et al. (2000) reported a similar developmental increase in GluR1 levels between P2 and P10 and P10 and P35, which remained stable into adulthood. A detailed timeline of changes in GluR1 levels during pre-adolescent development shows a peak sometime after P18, where levels then stabilize into adulthood. This developmental timeline coincides with a period of hippocampal

remodeling (reviewed in Holahan et al., 2019), indicating that GluR1 levels may somehow contribute to this remodeling and facilitate the transition from juvenile to adult-like cognitive functions.

We noted a distinct pattern of GluR1 staining on cells within the dentate gyrus that more or less paralleled the overall protein levels in the hippocampus. The regional expression of GluR1 within the hippocampus has been reported to vary dramatically during postnatal development. From P0 to P5, GluR1 is enriched in apical dendrites and the soma of pyramidal cells in the CA2, CA3, and subiculum while the dentate gyrus and CA1 pyramidal cells show low GluR1 expression (Ryo et al., 1993). By P14, GluR1 levels remain elevated in the CA2 and CA3 regions with increasing levels in the CA1, the dentate gyrus, and the subiculum/entorhinal cortex (Ibaraki et al., 1999). After P21, GluR1 levels were highest in the CA1, followed by the dentate gyrus, the CA2 and CA3 regions (Martin et al., 1998; Palomero-Gallagher et al., 2003). The strata of the CA1 also show differential distribution of GluR1 with the stratum oriens labeling emerging before the stratum radiatum (Ryo et al., 1993; Martin et al., 1998). This aligns with the later maturation of the dendritic system, including the branching of apical dendrite lateral fibers into the stratum radiatum and preterminal fibers branching in the stratum lacunosum between P15 and P24. These morphological events coincide with the development of synaptic transmission within the CA1 during which time LTP within this region increases substantially (Pokorný and Yamamoto, 1981; Ryo et al., 1993; Martin et al., 1998).

In the present work, GluR1 levels were significantly lower at P50 than P30. This is in line with previous investigations reporting a similar peak in GluR1 during early development, followed by a decrease into adulthood (Pellegrini-Giampietro et al., 1991, 1992a; Durand and Zukin, 1993; Martin et al., 1998; Ibaraki et al., 1999; Bian et al., 2012; Blair et al., 2013; McHail

and Dumas, 2015). After the third postnatal week, GluR3 expression overtakes and replaces GluR1 expression, which may serve to explain the decrease in GluR1 levels observed in adults (Durand and Zukin, 1993; Martin et al., 1998; Blair et al., 2013; McHail and Dumas, 2015).

Levels of GluR2 are low compared to GluR1 during postnatal development, but high levels have been noted during the first postnatal week (Monyer et al., 1991; Wisden and Seeburg, 1993; Isaac et al., 2007). The developmental trajectory of GluR2 has shown an incremental increase from P0 to P15 with adult-levels reached by P35 (Sans et al., 2000; Pandey et al., 2015). Results from our study indicate that GluR2 protein reached adult levels around P26-30. Other reports have shown that almost 96% of AMPAR contained the GluR2 subunit by P20 (Pickard et al. (2000). Assessment of GluR2 mRNA expression showed peak levels around P14 (Pellegrini-Giampietro et al. (1991) and Durand and Zukin (1993)), and it is possible that protein reaches adult levels shortly after mRNA peaks, following a series of post-translational modifications (Liu et al., 2016).

Between P7 and P35, GluR2 levels increase in the dentate gyrus and are almost exclusively expressed in mature granule cells (Standley et al., 1995; Hagihara et al., 2011). GluR2 levels within the CA1 and CA3 are slightly lower relative to the dentate gyrus from P0 to P21, after which they begin to increase, reaching similar levels as the dentate gyrus by adulthood (Pellegrini-Giampietro et al., 1992a; Hagihara et al., 2011). Within the CA3, GluR2 subunits are localized to postsynaptic densities on thorny excrescences within the stratum lucidum and on distal dendritic spines and shafts within the strata radiatum and lacunosum moleculare. This distribution suggests that both the excitatory mossy fiber input to CA3 and the associational/commissural input to the stratum radiatum are partly mediated by GluR2 (Siegel et al., 1995).

The developmentally-associated changes in GluR1 and GluR2 levels may be a function of developmentally-linked changes in the function of synaptic regulatory proteins. The membrane-associated guanylate kinases (MAGUKs) associate with the C-terminus and function as anchor proteins to stabilize AMPAr at the synapse. They also contribute to synaptic plasticity mechanisms through the insertion of AMPAr (Petrulia et al., 2005; Henley and Wilkinson, 2016). The PSD-95 group, including PSD-95, PSD-93, SAP102, and SAP97, are the most widely studied MAGUKs, of which PSD-95 and SAP97 interact with and regulate subunit insertion at the postsynaptic density (Iwakura et al., 2001; Schnell et al., 2002; Tomita et al., 2003; Petrulia et al., 2005; Henley and Wilkinson, 2016). Their developmental timeline has been shown to correlate with that of the GluR1 and GluR2 levels; PSD-95 and SAP97 levels first emerge around P10 and significantly increase up to P35 (Sans et al., 2000; Petrulia et al., 2005; Bian et al., 2012; Pandey et al., 2015). Transmembrane AMPA receptor regulatory proteins (TARPs), such as stargazin, also function to modify and regulate AMPAr density at the synapse throughout development by facilitating the binding of AMPA with MAGUKs (Petrulia et al., 2005). They, too, show a developmental pattern that parallels GluR1 and GluR2; Blair et al. (2013) reported a significant increase in TARP from P17-19 to P22-24.

The development patterns of GluR1 and GluR2 coincide with the emergence of spatial processing capabilities, with multiple reports citing improvements on hippocampal-dependent memory tasks during this period (Rudy et al., 1987; Langston et al., 2010; Rainecki et al., 2010; Akers et al., 2012; Comba et al., 2015). These improved functions may be attributable to increased GluR1 and GluR2 levels given their contributions to LTP and synaptic plasticity. AMPAr insertion into the synapse during early development is initially low, resulting in synapses possessing mainly NMDAr, referred to as “silent synapses” (Rumpel et al., 1998; Liao

et al., 1999; McHail and Dumas, 2015). Within the first two postnatal weeks, AMPAR insertion increases, reducing the number of silent synapses, and facilitating LTP expression and synaptic strengthening, largely mediated by the phosphorylation of GluR1, leading to more GluR1 at the synapse (Isaac et al., 1995; Durand et al., 1996; Liao et al., 1999). This cycle of LTP expression/GluR1 insertion, coupled with the increased neurogenesis and synaptogenesis observed during this time of hippocampal remodeling, would likely saturate the synapse with GluR1 and decrease the threshold of LTP expression, increasing the strength and efficacy of the synapse, and resulting in the reported improvements in cognitive function.

Although GluR2 levels may not be directly involved with LTP expression, its regulatory role likely influences improvements in spatial processing seen during this time. GluR2-lacking AMPAR are largely Ca^{+2} -permeable and are present earlier in development. While an influx of Ca^{+2} into the cell is crucial for LTP and synaptic plasticity, it also renders the cell vulnerable to excitotoxicity and can oversaturate the synapse, leading to a net cessation of LTP expression and memory impairments if left unregulated (Barnes et al., 1994; Shimshek et al., 2006). Chronic neuronal excitation also leads to a decrease in excitatory synaptic transmission, known as synaptic downscaling (Siddoway et al., 2014). As such, an increase in GluR2 levels may help regulate Ca^{+2} influx via synaptic scaling, preventing oversaturation of LTP that may occur during this developmental period as a result of increased GluR1 levels. This would allow for a preservation of encoded information, while allowing for the continued strengthening of the synapse, and the reported improvements in cognitive function.

The pattern of GluR1 and GluR2 development within the ACC is similar to what has been found in previous investigations showing stagnated levels within the neocortex during this time (Pellegrini-Giampietro et al., 1991, 1992b; Orlandi et al., 2011). Despite this lack of

developmentally-linked changes in ACC GluR1 and GluR2 levels during the P18 – P50 period, GluR1 and GluR2 levels do show changes from P5 to P20, suggesting that the subunits within the ACC undergo changes earlier in postnatal development relative to the hippocampus (Pellegrini-Giampietro et al., 1991, 1992b; Orlandi et al., 2011). Additionally, by the third postnatal week, the cortical layers in the dorsal anterior cingulate have fully matured and the number of medial prefrontal cortex (mPFC) neurons have stabilized (van Eden and Uylings, 1985; Juraska and Willing, 2017). Despite this, the mPFC continues to show developmental changes including an increase in synaptic boutons from P35 to 45 (Drzewiecki et al., 2016), a spike in basal and apical dendritic spine density at P35 (Juraska and Willing, 2017), and a maturation of pre- and post-synaptic markers, like PSD-95 and synaptophysin, by P50 (Pinto et al., 2013; Testen et al., 2019). As the timeline used in this investigation falls between the timeline of this bimodal developmental pattern, the changes in GluR1 and GluR2 may not be detected at this time.

5. Conclusion

The distribution of GluR1 and GluR2 AMPAr subunits within the hippocampus and ACC from P18 - 30 were quantified and compared to adult (P50) levels. This allowed for the assessment of hippocampal and cortical cellular mechanisms associated with periods of remodeling in both regions. Hippocampal GluR1 and GluR2 levels showed variability throughout the observed developmental timepoints, reaching adult levels by P30. GluR1 and GluR2 levels within the ACC showed little variation during this time, suggesting a developmental timeline of maturation occurring earlier in postnatal development. The early development of the ACC may provide the basic “machinery” necessary to support cortical remodeling that might subserve remote memory function. Once the hippocampus undergoes remodeling and stabilizes its cellular mechanisms, an appropriate link would be made between these two structures allowing for the transfer of memory representations. Indeed, reports have shown the ability of P18 – P20 rats (Tzakis and Holahan, under review) and mice (Guskjolen et al., 2017) to form remote spatial memories that might rely on a functional coupling between the hippocampus and ACC. Given that the this hippocampal-ACC circuitry is highly influenced by experience, the next step would be to characterize GluR1 and GluR2 levels following a spatial remote memory task.

References

- Akers KG, Arruda-Carvalho M, Josselyn SA, Frankland PW (2012) Ontogeny of contextual fear memory formation, specificity, and persistence in mice. *Learn Mem* 19:598–604.
- Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124:319–335.
- Amaral DG, Dent JA (1981) Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions. *J Comp Neurol* 195:51–86.
- Barnes CA, Jung MW, McNaughton BL, Korol DL, Andreasson K, Worley PF (1994) LTP saturation and spatial learning disruption: Effects of task variables and saturation levels. *J Neurosci* 14:5793–5806.
- Barria A, Muller D, Derkach V, Griffith LC, Soderling TR (1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* (80) 276:2042–2045.
- Bayer SA (1980) Development of the hippocampal region in the rat I. Neurogenesis examined with 3H-thymidine autoradiography. *J Comp Neurol* 190:87–114.
- Bian C, Zhu K, Guo Q, Xiong Y, Cai W, Zhang J (2012) Sex differences and synchronous development of steroid receptor coactivator-1 and synaptic proteins in the hippocampus of postnatal female and male C57BL/6 mice. *Steroids* 77:149–156.

- Blair MG, Nguyen NNQ, Albani SH, L'Etoile MM, Andrawis MM, Owen LM, Oliveira RF, Johnson MW, Purvis DL, Sanders EM, Stoneham ET, Xu H, Dumas TC (2013) Developmental changes in structural and functional properties of hippocampal AMPARs parallels the emergence of deliberative spatial navigation in juvenile rats. *J Neurosci* 33:12218–12228.
- Bliss TVP, Gardner-Medwin AR (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. *J Physiol* 232:357–374.
- Bontempi B, Laurent-Demir C, Destrade C, Jaffard R (1999) Time-dependent reorganization of brain circuitry underlying long-term memory storage. *Nature* 400:671–675.
- Broadbent NJ, Squire LR, Clark RE (2004) Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci U S A* 101:14515–14520.
- Comba R, Gervais N, Mumby D, Holahan M (2015) Emergence of spatial behavioral function and associated mossy fiber connectivity and c-Fos labeling patterns in the hippocampus of rats. *F1000Research* 4.
- Curlik DM, DiFeo G, Shors TJ (2014) Preparing for adulthood: Thousands upon thousands of new cells are born in the hippocampus during puberty, and most survive with effortful learning. *Front Neurosci* 8.
- Dingledine R, Borges K, Bowie D, Traynelis S, Traynelis SF (1999) The Glutamate Receptor Ion Channels Allosteric modulation of NMDA receptors View project The Glutamate Receptor Ion Channels. *Pharmacol Rev* 51:7–61.

- Drzewiecki CM, Willing J, Juraska JM (2016) Synaptic number changes in the medial prefrontal cortex across adolescence in male and female rats: A role for pubertal onset. *Synapse* 70:361–368.
- Dumas TC (2012) Postnatal alterations in induction threshold and expression magnitude of long-term potentiation and long-term depression at hippocampal synapses. *Hippocampus* 22:188–199.
- Durand GM, Kovalchuk Y, Konnerth A (1996) Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* 381:71–75.
- Durand GM, Zukin RS (1993) Developmental Regulation of mRNAs Encoding Rat Brain Kainate/AMPA Receptors: A Northern Analysis Study. *J Neurochem* 61:2239–2246.
- Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nat Neurosci* 6:136–143.
- Fox K, Stryker M (2017) Integrating Hebbian and homeostatic plasticity: Introduction. *Philos Trans R Soc B Biol Sci* 372.
- Frankland PW, Bontempi B (2005) The organization of recent and remote memories. *Nat Rev Neurosci* 6:119–130.
- Frankland PW, Bontempi B (2006) Fast track to the medial prefrontal cortex. *Proc Natl Acad Sci U S A* 103:509–510.
- Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ (2004) The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory. *Science* (80) 304:881–883.
- Gaarskjaer FB (1985) The development of the dentate area and the hippocampal mossy fiber projection of the rat. *J Comp Neurol* 241:154–170.

- Gaarskjaer FB (1986) The organization and development of the hippocampal mossy fiber system. *Brain Res* 396:335–357.
- Gainey MA, Hurvitz-Wolff JR, Lambo ME, Turrigiano GG (2009) Synaptic scaling requires the GluR2 subunit of the AMPA receptor. *J Neurosci* 29:6479–6489.
- Guskjolen A, Josselyn SA, Frankland PW (2017) Age-dependent changes in spatial memory retention and flexibility in mice. *Neurobiol Learn Mem* 143:59–66.
- Hagihara H, Ohira K, Toyama K, Miyakawa T (2011) Expression of the AMPA receptor subunits GluR1 and GluR2 is associated with granule cell maturation in the dentate gyrus. *Front Neurosci* 5.
- Henley JM, Wilkinson KA (2016) Synaptic AMPA receptor composition in development, plasticity and disease. *Nat Rev Neurosci* 17:337–350.
- Holahan MR, Honegger KS, Routtenberg A (2007) Expansion and retraction of hippocampal mossy fibers during postweaning development: Strain-specific effects of NMDA receptor blockade. *Hippocampus* 17:58–67.
- Holahan MR, Rekart JL, Sandoval J, Routtenberg A (2006) Spatial learning induces presynaptic structural remodeling in the hippocampal mossy fiber system of two rat strains. *Hippocampus* 16:560–570.
- Holahan MR, Tzakis N, Oliveira FA (2019) Developmental Aspects of Glucose and Calcium Availability on the Persistence of Memory Function Over the Lifespan. *Front Aging Neurosci* 11.
- Hollmann M, Heinemann S (1994) Cloned Glutamate Receptors. *Annu Rev Neurosci* 17:31–108.

- Ibaraki K, Otsu Y, Nawa H (1999) A novel two-site enzyme immunoassay reveals the regional distributions of and developmental changes in GluR1 and NMDAR1 protein contents in the rat brain. *J Neurochem* 73:408–417.
- Isaac JTR, Ashby M, McBain CJ (2007) The Role of the GluR2 Subunit in AMPA Receptor Function and Synaptic Plasticity. *Neuron* 54:859–871.
- Isaac JTR, Nicoll RA, Malenka RC (1995) Evidence for silent synapses: Implications for the expression of LTP. *Neuron* 15:427–434.
- Iwakura Y, Nagano T, Kawamura M, Horikawa H, Ibaraki K, Takei N, Nawa H (2001) N-methyl-D-aspartate-induced α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor down-regulation involves interaction of the carboxyl terminus of GluR2/3 with Pick1: Ligand-binding studies using Sindbis vectors carrying AMPA receptor de. *J Biol Chem* 276:40025–40032.
- Juraska JM, Willing J (2017) Pubertal onset as a critical transition for neural development and cognition. *Brain Res* 1654:87–94.
- Langston RF, Ainge JA, Couey JJ, Canto CB, Bjerknes TL, Witter MP, Moser EI, Moser MB (2010) Development of the spatial representation system in the rat. *Science* (80) 328:1576–1580.
- Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405:955–959.

- Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* 112:631–643.
- Lee HK, Takamiya K, He K, Song L, Huganir RL (2010) Specific roles of AMPA receptor subunit GluR1 (GluA1) phosphorylation sites in regulating synaptic plasticity in the CA1 region of hippocampus. *J Neurophysiol* 103:479–489.
- Liao D, Zhang X, O'Brien R, Ehlers MD, Huganir RL (1999) Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nat Neurosci* 2:37–43.
- Liu Y, Beyer A, Aebersold R (2016) On the Dependency of Cellular Protein Levels on mRNA Abundance. *Cell* 165:535–550.
- Lopez J, Herbeaux K, Cosquer B, Engeln M, Muller C, Lazarus C, Kelche C, Bontempi B, Cassel JC, de Vasconcelos AP (2012) Context-dependent modulation of hippocampal and cortical recruitment during remote spatial memory retrieval. *Hippocampus* 22:827–841.
- Malinow R (2003) AMPA receptor trafficking and long-term potentiation. *Philos Trans R Soc B Biol Sci* 358:707–714.
- Maren S, Tocco G, Standley S, Baudry M, Thompson RF (1993) Postsynaptic factors in the expression of long-term potentiation (LTP): Increased glutamate receptor binding following LTP induction in vivo. *Proc Natl Acad Sci U S A* 90:9654–9658.
- Martin LJ, Furuta A, Blackstone CD (1998) AMPA receptor protein in developing rat brain: Glutamate receptor-1 expression and localization change at regional, cellular, and subcellular levels with maturation. *Neuroscience* 83:917–928.

- Maviel T, Durkin TP, Menzaghi F, Bontempi B (2004) Sites of neocortical reorganization critical for remote spatial memory. *Science* (80) 305:96–99.
- McHail DG, Dumas TC (2015) Multiple forms of metaplasticity at a single hippocampal synapse during late postnatal development. *Dev Cogn Neurosci* 12:145–154.
- Monyer H, Seeburg PH, Wisden W (1991) Glutamate-operated channels: Developmentally early and mature forms arise by alternative splicing. *Neuron* 6:799–810.
- Morris RGM, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 319:774–776.
- Morris RGM, Garrud P, Rawlins JNP, O’Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. *Nature* 297:681–683.
- Murphy KM, Tcharnaia L, Beshara SP, Jones DG (2012) Cortical development of AMPA receptor trafficking proteins. *Front Mol Neurosci* 5.
- Orlandi C, la Via L, Bonini D, Mora C, Russo I, Barbon A, Barlati S (2011) AMPA receptor regulation at the mRNA and protein level in rat primary cortical cultures. *PLoS One* 6:e25350.
- Palomero-Gallagher N, Bidmon HJ, Zilles K (2003) AMPA, kainate, and NMDA receptor densities in the hippocampus of untreated male rats and females in estrus and diestrus. *J Comp Neurol* 459:468–474.
- Pandey SP, Rai R, Gaur P, Prasad S (2015) Development- and age-related alterations in the expression of AMPA receptor subunit GluR2 and its trafficking proteins in the hippocampus of male mouse brain. *Biogerontology* 16:317–328.

- Pellegrini-Giampietro DE, Bennett MVL, Zukin RS (1991) Differential expression of three glutamate receptor genes in developing rat brain: An in situ hybridization study. *Proc Natl Acad Sci U S A* 88:4157–4161.
- Pellegrini-Giampietro DE, Bennett MVL, Zukin RS (1992a) Are Ca²⁺-permeable kainate/AMPA receptors more abundant in immature brain? *Neurosci Lett* 144:65–69.
- Pellegrini-Giampietro DE, Zukin RS, Bennett MVL, Cho S, Pulsinelli WA (1992b) Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats. *Proc Natl Acad Sci U S A* 89:10499–10503.
- Petralia RS, Sans N, Wang YX, Wenthold RJ (2005) Ontogeny of postsynaptic density proteins at glutamatergic synapses. *Mol Cell Neurosci* 29:436–452.
- Pickard L, Noel J, Henley JM, Collingridge GL, Molnar E (2000) Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. *J Neurosci* 20:7922–7931.
- Pinto JGA, Jones DG, Murphy KM (2013) Comparing development of synaptic proteins in rat visual, somatosensory, and frontal cortex. *Front Neural Circuits* 7.
- Pokorný J, Yamamoto T (1981) Postnatal ontogenesis of hippocampal CA1 area in rats. I. Development of dendritic arborisation in pyramidal neurons. *Brain Res Bull* 7:113–120.
- Raineki C, Holman PJ, Debiec J, Bugg M, Beasley A, Sullivan RM (2010) Functional emergence of the hippocampus in context fear learning in infant rats. *Hippocampus* 20:1037–1046.
- Riedel G, Micheau J, Lam AGM, Roloff EVL, Martin SJ, Bridge H, De Hoz L, Poeschel B, McCulloch J, Morris RGM (1999) Reversible neural inactivation reveals hippocampal participation in several memory processes. *Nat Neurosci* 2:898–905.

- Rudy JW, Stadler-Morris S, Albert P (1987) Ontogeny of Spatial Navigation Behaviors in the Rat: Dissociation of “Proximal”-and “Distal”-Cue-Based Behaviors. *Behav Neurosci* 101:62–73.
- Rumpel S, Hatt H, Gottmann K (1998) Silent synapses in the developing rat visual cortex: Evidence for postsynaptic expression of synaptic plasticity. *J Neurosci* 18:8863–8874.
- Ryo Y, Miyawaki A, Furuichi T, Mikoshiba K (1993) Expression of the metabotropic glutamate receptor mGluR1 α and the ionotropic glutamate receptor GluR1 in the brain during the postnatal development of normal mouse and in the cerebellum from mutant mice. *J Neurosci Res* 36:19–32.
- Sanderson DJ, Good MA, Seeburg PH, Sprengel R, Rawlins JNP, Bannerman DM (2008) The role of the GluR-A (GluR1) AMPA receptor subunit in learning and memory. *Prog Brain Res* 169:159–178.
- Sans N, Petralia RS, Wang YX, Blahos J, Hell JW, Wenthold RJ (2000) A developmental change in NMDA receptor-associated proteins at hippocampal synapses. *J Neurosci* 20:1260–1271.
- Schnell E, Sizemore M, Karimzadegan S, Chen L, Brecht DS, Nicoll RA (2002) Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc Natl Acad Sci U S A* 99:13902–13907.
- Schoepfer R, Monyer H, Sommer B, Wisden W, Sprengel R, Kuner T, Lomeli H, Herb A, Köhler M, Burnashev N, Günther W, Ruppersberg P, Seeburg P (1994) Molecular biology of glutamate receptors. *Prog Neurobiol* 42:353–357.

- Shimshek DR, Jensen V, Celikel T, Geng Y, Schupp B, Bus T, Mack V, Marx V, Hvalby Ø, Seeburg PH, Sprengel R (2006) Forebrain-specific glutamate receptor B deletion impairs spatial memory but not hippocampal field long-term potentiation. *J Neurosci* 26:8428–8440.
- Siddoway B, Hou H, Xia H (2014) Molecular mechanisms of homeostatic synaptic downscaling. *Neuropharmacology* 78:38–44.
- Siegel SJ, Janssen WG, Tullai JW, Rogers SW, Moran T, Heinemann SF, Morrison JH (1995) Distribution of the excitatory amino acid receptor subunits GLUR2(4) in monkey hippocampus and colocalization with subunits GLUR5-7 and NMDAR1. *J Neurosci* 15:2707–2719.
- Standley S, Tocco G, Tourigny MF, Massicotte G, Thompson RF, Baudry M (1995) Developmental changes in α -amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor properties and expression in the rat hippocampal formation. *Neuroscience* 67:881–892.
- Tanaka H, Grooms SY, Bennett MVL, Zukin RS (2000) The AMPAR subunit GluR2: Still front and center-stage. *Brain Res* 886:190–207.
- Teixeira CM, Pomedli SR, Maei HR, Kee N, Frankland PW (2006) Involvement of the anterior cingulate cortex in the expression of remote spatial memory. *J Neurosci* 26:7555–7564.
- Testen A, Ali M, Sexton HG, Hodges S, Dubester K, Reissner KJ, Swartzwelder HS, Risher ML (2019) Region-Specific Differences in Morphometric Features and Synaptic Colocalization of Astrocytes During Development. *Neuroscience* 400:98–109.
- Tomita S, Chen L, Kawasaki Y, Petralia RS, Wenthold RJ, Nicoll RA, Brecht DS (2003) Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. *J Cell Biol* 161:805–816.

- Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391:892–896.
- Tzakis N, Holahan MR (under review). Factors that influence the processing of juvenile-acquired spatial memories in rats during hippocampal development. Manuscript submitted for publication.
- van Eden CG, Uylings HBM (1985) Cytoarchitectonic development of the prefrontal cortex in the rat. *J Comp Neurol* 241:253–267.
- Wartman BC, Gabel J, Holahan MR (2014) Inactivation of the anterior cingulate reveals enhanced reliance on cortical networks for remote spatial memory retrieval after sequential memory processing. *PLoS One* 9.
- Wartman BC, Holahan MR (2013) The use of sequential hippocampal-dependent and -non-dependent tasks to study the activation profile of the anterior cingulate cortex during recent and remote memory tests. *Neurobiol Learn Mem* 106:334–342.
- Wartman BC, Holahan MR (2014) The impact of multiple memory formation on dendritic complexity in the hippocampus and anterior cingulate cortex assessed at recent and remote time points. *Front Behav Neurosci* 8.
- Weible AP, Rowland DC, Monaghan CK, Wolfgang NT, Kentros CG (2012) Neural correlates of long-term object memory in the mouse anterior cingulate cortex. *J Neurosci* 32:5598–5608.
- Wiltgen BJ, Brown RAM, Talton LE, Silva AJ (2004) New circuits for old memories: The role of the neocortex in consolidation. *Neuron* 44:101–108.
- Wisden W, Seeburg PH (1993) Mammalian ionotropic glutamate receptors. *Curr Opin Neurobiol* 3:291–298.

- Wright A, Vissel B (2012) The essential role of AMPA receptor GluA2 subunit RNA editing in the normal and diseased brain. *Front Mol Neurosci* 5.
- Zhao C, Teng EM, Summers RG, Ming GL, Gage FH (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci* 26:3–11.
- Zhu JJ, Esteban JA, Hayashi Y, Malinow R (2000) Postnatal synaptic potentiation: Delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nat Neurosci* 3:1098–1106.

Chapter 4

Assessing the development of juvenile remote memory processing using an open field task and c-Fos staining in the hippocampus and anterior cingulate cortex

Nikolaos Tzakis, Tanisse Teale, Matthew R. Holahan
Department of Neuroscience

Manuscript submitted to: Hippocampus

Abstract

The emergence of complex cognitive processes during development coincides with a reorganization of connectivity within and between brain regions. The hippocampus is one such region that undergoes extensive postnatal morphological and cellular remodeling, which has been hypothesized to mediate the emergence of spatial memory processing. The current work investigated whether preadolescent spatial memories acquired during this period of hippocampal synaptic modification persisted beyond 24 hours and stabilized into the postadolescent period in cortical networks. Rats were exposed to an open field task for one, 30 min session on postnatal day (P) 16, 18, 20, 25, 30 or 50 and re-exposed for 30 min at either a recent (24 hours) or remote (3 weeks) timepoint. A shorter distance traveled during the test was reflective of an intact memory. Using this metric, P16, P18 and P20 rats showed no recent memory or remote memory. Intact recent and remote memories were noted at P25 and P50 while the P30 group showed an intact recent memory but a weak remote memory. In the hippocampus CA1 region, c-Fos levels were higher after the remote test than the recent test in the P16 – P20 groups but higher after the recent test than remote test in the P25 – P50 groups. c-Fos labeling in the ACC was higher in the remote-tested groups compared to the recent across all ages. GluR1 and GluR2 protein in the hippocampus was not associated with behavioral changes while GluR1 in the ACC was more closely associated with the remote memory test particularly in the P16 – P20 groups. Results suggest that spatial information processing and recent memory emerges around P20 and that remote memory function emerges around P25. From P16 – P20, hippocampal-cortical networks might be too active to allow these networks to store remote memories while after this time, the maturation of these networks allows for proper remote memory storage.

1. Introduction

During late preadolescent development, the hippocampus undergoes a series of cellular and morphological changes hypothesized to mediate the transition from juvenile-like to adult-like spatial memory processing (Holahan et al., 2019). From postnatal day (P) 15 to 18, the dentate gyrus (DG) shows an increase in neurogenesis followed by widespread mossy fiber connectivity changes between DG granule cells and CA3 pyramidal neurons (Altman and Das, 1965; Bayer, 1980; Amaral and Dent, 1981; Gaarskjaer, 1985, 1986; Holahan et al., 2006, 2007; Zhao et al., 2006; Curlik et al., 2014). These morphological changes correspond with improvements in performance on spatial memory tasks (Comba et al., 2015). While these data point to an association between hippocampal maturation and emergence of spatial processing function, it is not clear whether this hippocampal remodeling coincides with the remote processing and storage of spatial information through the strengthening of hippocampal-cortical connections.

One such cortical region connected with the hippocampus that contributes to the remote storage of information is the anterior cingulate cortex (ACC). Following remote memory tests, the ACC shows increased activity and structural changes, while inactivation of this region impairs performance on remote memory spatial tasks (Bontempi et al., 1999; Teixeira et al., 2006; Weible et al., 2012; Wartman and Holahan, 2014). During these tests of remote memory, hippocampal networks appear to become less prominent and the memory comes to rely on the intra-cortical connections, ultimately being encoded into remote stores within the ACC and associated cortical regions (Bontempi et al., 1999; Teixeira et al., 2006; Weible et al., 2012; Wartman et al., 2014).

The ACC is thought to undergo remodeling that either overlaps or is slightly later in development relative to the hippocampus. From approximately P24 to P30, there is a reduction in the colocalization of astrocytes with dendritic markers (Testen et al., 2019). Markers of pre- and post-synaptic maturation show prolonged development in the prefrontal cortical areas (including the ACC) that reach adult-like levels around P36 for the pre-synaptic index (starting around P29) and P30 for the post-synaptic index (starting around P22 (Pinto et al., 2013)). Levels of immediate early genes, c-Fos, *zif268* and *Arc* are elevated in P17 and P24 rats compared to adults (P80 (Jia et al., 2018)). An RNA-sequencing (RNA-seq) study of the rat medial PFC (mPFC) at five developmental time points revealed a change in gene expression patterns from regulation of network formation to maintenance around PND21 (Kroeze et al., 2018). The authors suggested that this change in gene expression may contribute to the formation of stable networks within the PFC that underlies the performance of cognitive and emotional tasks (Kroeze et al., 2018).

Fast, excitatory post-synaptic currents are mediated by synaptic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor activity. These receptors regulate remote potentiation (LTP), a prospective neural correlate of learning and memory, through persistent modifications, characterized by an increase in their binding affinity, number of binding sites, or a change in their ion channel kinetics (Maren et al., 1993; Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Andrásfalvy and Magee, 2004; Kessels and Malinow, 2009; Makino and Malinow, 2009; Penn et al., 2017; Shimshek et al., 2017). AMPARs are composed of four subunits with the majority of synaptic AMPARs within the hippocampal CA1 region composed of GluR1 and GluR2 heteromers (Henley and Wilkinson, 2016). The pattern of GluR1 and GluR2 expression throughout early postnatal development parallels the timeline of hippocampal remodeling and emergence of spatial memory function. GluR1 levels are initially higher than GluR2 and GluR1

levels continue to increase from P5 to P21, after which levels decline (Pellegrini-Giampietro et al., 1992; Pickard et al., 2000; Dumas, 2012; Blair et al., 2013; Henley and Wilkinson, 2016). Synaptic AMPAr continue to express GluR2 throughout early postnatal development, such that by P14, almost all AMPAr heterotetramers are comprised of GluR2, rendering these AMPAr Ca^{+2} -impermeable (Pellegrini-Giampietro et al., 1992; Pickard et al., 2000; Henley and Wilkinson, 2016).

In the current investigation, rats were exposed (trained) to an open field task at different developmental timepoints (P16, 18, 20, 25, 30, or 50) and re-exposed (tested) either 24 hours or 3 weeks following the first exposure. The objective was to assess the transition from juvenile- to adult-like memory processing on both recent and remote tests. To remedy potential sensorimotor confounds associated with the use of the water maze task (Vorhees and Williams, 2006; Sharma et al., 2010; Wartman et al., 2012; Comba et al., 2015), variants of the open field task have been used to assess spatial cognition (Dvorkin et al., 2008; Lipatova et al., 2015). For the purposes of this investigation, rats were placed into an open field surrounded by spatial cues for 30 minutes (training) and then reintroduced to the open field for another 30-minute trial (testing) either 24 hours or 3 weeks following the training trial. The difference in distance traveled between testing and training was used as a measure of spatial retention. Reduced exploratory behavior at the time of testing signified that the subject recognized the environment as familiar leading to habitation and less exploratory behavior. The use of the open field task to assess spatial memory was thought to complement findings on other spatial tasks used during development (reviewed by (Holahan et al., 2019)). Western blotting was also performed on the hippocampus and ACC to evaluate the variation in expression levels of GluR1 and GluR2 following open field testing to determine possible neural underpinnings that may correlate with the emergence of recent and

remote memory processing. c-Fos immunohistochemistry performed on the hippocampus and ACC allowed for the determination of recent activation in both regions following testing in order to assess the contribution of each region to recent and remote memory processes.

2. Materials and methods

2.1. Subjects

Male and female Long-Evans rats (LERs) were used throughout the experiment. Rats were born in the temperature and humidity-regulated animal facility at Carleton University. The day the pups were born was marked as P0. Pups were weaned on P18. Newly weaned rats were provided with solid food pellets soaked with some water and placed in a ceramic dish in the cage, along with a dish containing fortified water gel. Unsoaked food pellets were also placed in the cage. For all experiments, rats were group-housed in polycarbonate cages with a 12-hour light-dark cycle: lights on 8h00, lights off 20h00 with food and water provided *ad libitum*. Animal care was conducted in accordance with the Canadian Council on Animal Care (CCAC) guidelines and approved by the Carleton University Animal Care Committee.

2.2. Open field

Rats were divided into one of 6 groups, each undergoing training on a different developmental timepoint (P16 [males and females], 18 [males and females], 20 [males and females], 25 [males and females], 30 [males only], or 50 [males only]). On the training day, rats were placed into the open-field (3' x 3' x 3' box placed on the floor). The open field was surrounded by four, 6' tall banners placed around the outside of the box, serving as distal visual cues, which were present on all trials. Each rat was given one, thirty-minute training trial,

whereby they were individually placed into the box from the same location and allowed to explore freely. Total distance traveled and amount of time spent in the center and perimeter of the box were recorded. Following their trial, each rat was returned to their home cage and the box was cleaned with ethanol and allowed to dry before the next rat entered.

The rats were re-exposed to the box for a 24 hour (recent, P16: n = 4; P18: n = 5; P20: n = 7; P25: n = 7; P30: n = 9; P50: n = 8) or 3 week (remote, P16: n = 5; P18: n = 5; P20: n = 8; P25: n = 9; P30: n = 4; P50: n = 4) retention test following their training day. All rats were given one, thirty-minute test, whereby they were placed into the box from the same location and allowed to explore freely. Total distance traveled was recorded for all rats. After the test, rats were injected with 3 ml urethane until they were no longer responsive to a toe pinch. The box was cleaned with ethanol after each trial and dried before the next rat was tested.

2.3. Immunohistochemistry

Following urethane administration, rats were decapitated, and their brains were removed from the skull and hemisected. One hemisphere was immersion-fixed in 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS) overnight at 4°C. This solution was replaced with 30% sucrose in 0.1M PBS the following day and brains were stored at 4°C until sectioning. Brains were sectioned through the anterior cingulate cortex and the dorsal hippocampus at 60µm on a Leica CM1900 cryostat (Weztler, Germany). Sections were stored in a 0.1% sodium azide solution in 0.1M phosphate buffer (PB) at 4°C.

The ACC (P16: n = 4 for recent, n = 4 for remote; P18: n = 5 for recent, n = 5 for remote; P20: n = 4 for recent, n = 4 for remote; P25: n = 4 for recent, n = 5 for remote; P30: n = 4 for recent, n = 3 for remote; P50: n = 4 for recent, n = 3 for remote) and CA1 (P16: n = 4 for recent,

n = 4 for remote; P18: n = 4 for recent, n = 4 for remote; P20: n = 4 for recent, n = 4 for remote; P25: n = 4 for recent, n = 4 for remote; P30: n = 3 for recent, n = 4 for remote; P50: n = 3 for recent, n = 3 for remote) were stained for c-Fos. Sections were placed in phosphate-buffered saline with Triton X (PBS-TX) for three, 5-minute washes. They were then incubated in 3% hydrogen peroxide (H₂O₂) in PBS-TX for 15 minutes, followed by three, five-minute washes in PBS-TX. Sections were transferred to BLOXALL (Vector) for 10 minutes at room temperature, followed by one wash in PBS for 5 minutes. Incubation in the primary antibody mouse anti-c-Fos (1:1000; Phosphosolutions, Cat# 309-cFOS) occurred for 24 hours at room temperature. The tissue was washed in PBS-TX for three, 10-minute washes followed by a 2-hour incubation in the secondary antibody (anti-mouse biotinylated from Vector Laboratories, 1:500, Cat# BA-9200). Tissue was washed for three, 10-minute washes in PBS-TX before being placed into an ABC solution (Vector) for one hour. The tissue was rinsed in three, 5-minute washes using PBS before being placed into a DAB solution. Sections were then mounted on glass slides, dehydrated, and cover slipped.

c-Fos staining in each region was quantified using ImageJ software. Cells were counted within each region, and the cell count was divided by the counting area in order to determine the relative density of c-Fos activated cells within each region.

2.4. Western blotting

Tissue punches collected from the ACC (for GluR1: P16: n = 4 for recent, n = 5 for remote; P18: n = 5 for recent, n = 5 for remote; P20: n = 7 for recent, n = 5 for remote; P25: n = 6 for recent, n = 8 for remote; P30: n = 6 for recent, n = 4 for remote; P50: n = 8 for recent, n = 4 for remote; for GluR2: P16: n = 4 for recent, n = 5 for remote; P18: n = 5 for recent, n = 5 for

remote; P20: n = 7 for recent, n = 5 for remote; P25: n = 6 for recent, n = 9 for remote; P30: n = 7 for recent, n = 4 for remote; P50: n = 8 for recent, n = 4 for remote) and hippocampus (for GluR1: P16: n = 4 for recent, n = 5 for remote; P18: n = 5 for recent, n = 5 for remote; P20: n = 7 for recent, n = 8 for remote; P25: n = 7 for recent, n = 9 for remote; P30: n = 9 for recent, n = 4 for remote; P50: n = 7 for recent, n = 4 for remote; for GluR2: P16: n = 4 for recent, n = 5 for remote; P18: n = 5 for recent, n = 5 for remote; P20: n = 7 for recent, n = 8 for remote; P25: n = 6 for recent, n = 9 for remote; P30: n = 9 for recent, n = 4 for remote; P50: n = 8 for recent, n = 4 for remote) of the other hemisphere were used to for Western blotting quantification.

Whole cell lysates from collected from the hippocampus were homogenized in Radio Immuno Precipitation Assay (RIPA) buffer (50 mM Tris (pH 8.0), 150 mM sodium chloride, 0.1% sodium dodecyl sulphate (SDS), 0.5% sodium deoxycholate and 1% Triton X-100) mixed with 1 tablet of Complete Mini ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor (Roche Diagnostics, Laval, QC, Cat #11 836 170 001) per 10 mL of buffer and then sonicated for 10 seconds in ice cold water. The lysed cells were then centrifuged at 5000 RPM with a tabletop microcentrifuge for 10 minutes at 4°C. The supernatant was then extracted, and protein concentration was determined using bicinchoninic acid (BCA) method (Thermo Scientific). Following protein concentration determination, supernatant was placed in 5x loading buffer (containing 5% glycerol, 5% β -mercaptoethanol, 3% SDS and 0.05% bromophenol blue) and the protein was denatured when placed in a 5-minute heating block at a temperature of 105°C. Following this step, samples were then placed in a -20°C freezer until Western blotting commenced.

On day one of analysis, proteins were separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE gel (7.5%) containing the

separating buffer (370 mM Tris-base (pH 8.8), 3.5 mM SDS), and the stacking buffer (124 mM Tris-base (pH 6.8), 3.5 mM SDS), were placed in running buffer (25 mM Tris-base, 190 mM glycine, 3.5 mM SDS) and samples, along with the Precision Plus Protein™ Standards Dual Color (Bio-Rad, Hercules, CA, Cat#161-0374), were loaded into the Arcylamide gel (7.5 %) for molecular weight determination at 140 volts. After electrophoresis, proteins were transferred for one hour at 4°C at 100 volts in transfer buffer solution (25 mM Tris-base, 192 mM Glycine, 20% methanol), onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Cat#162-0177). Thereafter, membranes were dried overnight. The following day, membranes were reactivated using methanol and total protein load concentration was determined.

To determine total protein, after a brief methanol rinse (5 seconds), membranes were incubated in REVERT total protein solution for a period of 5 minutes followed by placement into a REVERT wash solution (6.7% Glacial Acetic Acid, 30% Methanol, in water) two times 2 minutes each. Membranes were then quickly rinsed with distilled water and imaged on a LI-COR Odyssey imaging system on the 700 channel for an exposure period of 2 minutes. Membranes were then rinsed immediately post imaging in tris buffered saline ((TBS) pH 7.5 (2 X 5min.)) buffer followed by being blocked with 0.5% fish gelatin (Sigma) in TBS for 60 minutes. Membranes were incubated with rabbit anti-GluR1 (1:1000; EMD Millipore, Cat# AB1504) or rabbit anti-GluR2 (1:4000; EMD Millipore, AB1768-I) overnight in 0.05% fish gelatin in TBS with 0.1% tween. Any unbound antibody was removed using 15 mL of TBS-T/membrane at room temperature three times five minutes each. Membranes were then incubated in infrared conjugate secondary (AlexaFluor 680 goat anti-rabbit; Invitrogen, Cat# AB175773) at a concentration of 1:4000 in 0.5% fish gelatin solution containing 0.2% tween and 0.01% SDS.

Membranes were then washed in TBS-T (1 X 5 minutes) followed by 2 X 5 minutes washes in TBS and read on a Licor Odyssey system on the 700-channel for 6 minutes.

Total protein expression from each sample on each membrane was determined using the total protein stain method in order to provide normalization across each specific membrane. To determine protein expression, bands at the appropriate molecular weight were determined with the Odyssey software. Presented data represents the ratio of target protein signal strength to normalized total protein signal strength.

2.5. Statistical analyses

Results from the open field test were analyzed using a fixed factor multivariate test (testing interval [remote vs recent] as the between-subjects factor and trial [training vs testing] as the repeated measures for each age group. An independent samples T-test was conducted to assess differences between trials. A fixed factor ANOVA (age and testing interval as the fixed factors and difference scores as the dependent variable) was conducted on the difference score data. The ratio of distance traveled in center to total distance traveled was analyzed using a fixed factor ANOVA (trial as the between -subjects factor and time bin as the repeated measures) for each age group and testing interval.

A fixed factor ANOVA was conducted on the immunohistochemical and Western blotting data (age and testing interval as the fixed factors and cell count/signal strength as the dependent variable). A one-way ANOVA and Tukey post-hoc comparison were used to assess differences in age and/or testing interval when a significant age x testing interval was present. Data are graphically represented as mean $\pm$ SEM.

3. Results

3.1. Open field task

Total distance traveled during the open field task for rats in each age group is shown in Figure 1. The pattern of results shows a linear decrease across age in distance traveled at the 24-hour, recent test that plateaued at P25 and remained similar to P50. During the remote test, distance traveled increased from P16 to P18 then showed a substantial decrease at P20 followed by a significant reduction at P25 then an upswing at P30 and a decreased exploration in the P50 group.

Distance traveled data for the P16 group is shown in Figure 1A. A fixed factor repeated measures ANOVA revealed no main effect of trial ($F_{(1, 7)} = 2.153, p = 0.186$), testing interval ($F_{(1, 7)} = 0.303, p = 0.599$) nor trial by testing interval interaction ($F_{(1, 7)} = 0.111, p = 0.749$). An independent samples T-test revealed no difference between the total distance traveled during testing and training for this age group at 24 hours ($t_{(6)} = -0.531, p = 0.614$) or 3 weeks ($t_{(8)} = -1.368, p = 0.208$).

Distance traveled for the P18 group is shown in Figure 1B. A fixed factor ANOVA revealed main effects of trial ($F_{(1, 8)} = 12.210, p < 0.01$), but no main effect of testing interval ($F_{(1, 8)} = 0.062, p = 0.810$) or trial by testing interval interaction ($F_{(1, 8)} = 1.203, p = 0.305$). An independent samples T-test revealed no difference between the total distance traveled during testing and training for this age group at 24 hours ($t_{(8)} = -1.248, p = 0.247$) or 3 weeks ($t_{(8)} = -2.209, p = 0.058$).

Distance traveled for the P20 group is shown in Figure 1C. A fixed factor ANOVA revealed main effects of trial ($F_{(1, 13)} = 8.211, p < 0.05$) and testing interval ($F_{(1, 13)} = 10.851, p < 0.01$) but no significant trial by testing interval interaction ($F_{(1, 13)} = 0.689, p = 0.422$). An

independent samples T-test revealed that the total distance traveled during testing was significantly higher than during training for the P20:recent group ($t_{(12)} = -2.568, p < 0.05$) but not the P20:remote groups ($t_{(14)} = -2.568, p = 0.053$).

Distance traveled for the P25 group is shown in Figure 1D. A fixed factor ANOVA revealed no main effects of trial ($F_{(1, 14)} = 0.846, p = 0.373$), testing interval ($F_{(1, 14)} = 0.164, p = 0.691$) or trial by testing interval interaction ($F_{(1, 14)} = 0.227, p = 0.641$). An independent samples T-test revealed no difference between the total distance traveled during testing and training for this age group at 24 hours ($t_{(12)} = 0.350, p = 0.733$) or 3 weeks ($t_{(16)} = 1.044, p = 0.312$).

Distance traveled for the P30 group is shown in Figure 1E. A fixed factor ANOVA revealed no main effects of trial ($F_{(1, 11)} = 1.464, p = 0.252$), testing interval ($F_{(1, 11)} = 4.453, p = 0.059$), or trial by testing interval interaction ($F_{(1, 11)} = 0.189, p = 0.672$). An independent samples T-test revealed no difference between the total distance traveled during testing and training for this age group at 24 hours ($t_{(16)} = -0.545, p = 0.593$) or 3 weeks ($t_{(6)} = -0.950, p = 0.379$).

Distance traveled for the P50 group is shown in Figure 1F. A fixed factor ANOVA revealed no main effects of trial ($F_{(1, 10)} = 0.474, p = 0.507$) and testing interval ($F_{(1, 10)} = 0.629, p = 0.446$), or trial by testing interval interaction ($F_{(1, 10)} = 1.253, p = 0.289$). An independent samples T-test revealed no difference between the total distance traveled during testing and training for this age group at 24 hours ($t_{(14)} = -0.118, p = 0.908$) or 3 weeks ($t_{(6)} = 0.598, p = 0.572$).

The difference between distance traveled during testing and training for each age is shown in Figure 2. A fixed factor ANOVA revealed a main effect of age group ($F_{(5, 63)} = 3.468, p < 0.01$), but no main effect of testing interval ($F_{(1, 63)} = 0.578, p = 0.450$) or age group by testing

interval interaction ($F_{(5, 63)} = 0.484, p = 0.787$). An independent samples t-test revealed significant differences between P20:recent and P25:recent ($t_{(12)} = 2.248, p < 0.05$).

The ratio of distance traveled in the center of the field to total distance traveled between training and testing, analyzed across three 10-minute time increments (time bins: 0-10 minutes, 10-20 minutes, 20-30 minutes), for each age group is shown in Figure 3. These data show that the P16, 18, and 20 groups increased the distance traveled in the center between training and testing within the 24 hour and 3 week intervals, while the P25 and 30 groups did not show any change at either interval, and the P50 group spent more time in the center compared to all other age groups.

The ratios for the P16 group are shown in Figure 3A and 3B. A fixed factor ANOVA revealed no main effect of time bin ($F_{(2, 12)} = 0.775, p = 0.482$), trial ($F_{(1, 6)} = 0.775, p = 0.482$), or time bin by trial interaction ($F_{(2, 12)} = 0.778, p = 0.481$) for the recent group, and no main effect of time bin ($F_{(2, 16)} = 1.375, p = 0.281$), trial ($F_{(1, 8)} = 4.394, p = 0.069$), or time bin by trial interaction ($F_{(2, 16)} = 0.238, p = 0.791$) for the remote group.

The ratios for the P18 group are shown in Figure 3C and 3D. A fixed factor ANOVA revealed a main effect of time bin ($F_{(2, 16)} = 8.362, p < 0.01$), but no main effect of trial ($F_{(1, 8)} = 1.881, p = 0.207$), or time bin by trial interaction ($F_{(2, 16)} = 2.553, p = 0.109$) for the recent group, and no main effect of time bin ($F_{(2, 16)} = 1.158, p = 0.339$), trial ($F_{(1, 8)} = 2.635, p = 0.143$), or time bin by trial interaction ($F_{(2, 16)} = 0.471, p = 0.633$) for the remote group.

The ratios for the P20 group are shown in Figure 3E and 3F. A fixed factor ANOVA revealed no main effect of time bin ($F_{(2, 24)} = 6.688, p < 0.01$), trial ($F_{(1, 12)} = 0.373, p = 0.553$), or time bin by trial interaction ($F_{(2, 24)} = 1.250, p = 0.305$) for the recent group, and no main effect

of time bin ($F_{(2, 28)} = 1.776, p = 0.188$), trial ($F_{(1, 14)} = 0.006, p = 0.938$), or time bin by trial interaction ($F_{(2, 28)} = 0.283, p = 0.755$) for the remote group.

The ratios for the P25 group are shown in Figure 3G and 3H. A fixed factor ANOVA revealed no main effect of time bin ($F_{(2, 24)} = 3.226, p = 0.057$), but no main effect of trial ($F_{(1, 12)} = 0.337, p = 0.572$), or time bin by trial interaction ($F_{(2, 24)} = 1.467, p = 0.251$) for the recent group, and no main effect of time bin ($F_{(2, 32)} = 0.017, p = 0.984$), trial ($F_{(1, 16)} = 0.003, p = 0.960$), or time bin by trial interaction ($F_{(2, 32)} = 1.184, p = 0.319$) for the remote group.

The ratios for the P30 group are shown in Figure 3I and 3J. A fixed factor ANOVA revealed a main effect of time bin ($F_{(2, 32)} = 10.407, p < 0.001$), but no main effect of trial ($F_{(1, 16)} = 1.316, p = 0.268$), or time bin by trial interaction ($F_{(2, 32)} = 1.324, p = 0.280$) for the recent group, and no main effect of time bin ($F_{(2, 12)} = 0.156, p = 0.857$), trial ($F_{(1, 6)} = 0.624, p = 0.460$), or time bin by trial interaction ($F_{(2, 12)} = 0.089, p = 0.916$) for the remote group.

The ratios for the P50 group are shown in Figure 3K and 3L. A fixed factor ANOVA revealed no main effect of time bin ($F_{(2, 28)} = 1.403, p = 0.263$), trial ($F_{(1, 14)} = 0.025, p = 0.876$), or time bin by trial interaction ($F_{(2, 28)} = 0.343, p = 0.713$) for the recent group, and no main effect of time bin ($F_{(2, 12)} = 0.955, p = 0.412$), trial ($F_{(1, 6)} = 0.130, p = 0.731$), or time bin by trial interaction ($F_{(2, 12)} = 2.262, p = 0.147$) for the remote group.

Figure 1. Total distance traveled during training and testing in the open field task. (A) P16, (B) P18, (C) P20; (*) indicates significant difference in total distance traveled between the training and testing trial ($p < 0.05$), (D) P25, (E) P30, (F) P50.

Figure 2. Difference scores showing the mean difference between distance traveled during testing and training trials for each age and testing group.

Figure 3. Ratio of the distance traveled in the center of the field to the total distance traveled during training and testing for each age group measured across 10 minute increments.

3.2. Immunohistochemistry

3.2.1. CA1

Representative examples of c-Fos staining in the CA1 region are shown in Figure 4. A fixed factor ANOVA on c-Fos staining in the CA1 (Fig 5) revealed a main effect of age ($F_{(5, 33)} = 8.248, p < 0.001$), testing interval ($F_{(1, 33)} = 12.580, p < 0.01$), and age by testing interval interaction ($F_{(5, 33)} = 52.840, p < 0.001$). Post-hoc tests conducted on the recently and remotely tested groups revealed significantly more c-Fos staining in the remotely tested groups relative to the recently tested groups at P16, 18, and 20 ($p < 0.001$), and significantly more c-Fos staining in the recently tested groups relative to the remotely tested groups at P25 and 30 ($p < 0.01$). Post-hoc tests conducted on the recently tested groups revealed significantly more c-Fos staining in the P25, 30, and 50 age groups compared to the P18 age groups, in the P25 and 30 age groups compared to the P18 and 20 age groups, and in the P25 age group compared to the P16 age group ($p < 0.05$). Post-hoc tests conducted on the remotely tested groups revealed significantly more c-Fos staining in the P16, 18, and 20 age groups compared to the P25, 30, and 50 age groups ($p < 0.001$).

3.2.2. ACC

Representative c-Fos staining in the ACC is shown in Figure 6. A fixed-factor ANOVA on c-Fos staining in the ACC (Fig 7) revealed a main effect of age ($F_{(5, 37)} = 4.330, p < 0.01$), and testing interval ($F_{(1, 37)} = 266.561, p < 0.001$), and a significant age by testing interval interaction ($F_{(5, 37)} = 3.428, p < 0.05$). Tukey's post-hoc tests conducted on the recently and remotely tested groups revealed significantly more c-Fos staining in the remotely tested groups relative to the recently tested groups across all ages ($p < 0.05$). Post-hoc tests conducted on the

remotely tested groups revealed significantly more c-Fos staining at P18 compared to the P30 and 50 age groups ($p < 0.05$).

A.

B.

P16

P50

Recent

Remote

Figure 4. (A) Representative image of the hippocampus stained with NeuN outlining the area of the CA1 used for cell counts. NeuN protein is found within the nucleus and perinuclear cytoplasm of neurons (Gusel'nikova and Korzhevsky, 2015). It was used to highlight the count region used for the c-Fos count. (B) Representative images of c-Fos levels in the CA1 region of the hippocampus following recent and remote testing for the P16 and 50 age groups.

Figure 5. c-Fos quantification in the CA1 region of the hippocampus. (!!) indicates significant difference between the P16, 18, and 20 age groups the P25, 30, and 50 age groups tested remotely, and between the recently tested and remotely tested groups within each age group ($p < 0.01$). (*) indicates significant difference between the P25 and 30 age groups and the P18, and 20 age groups tested recently, and between the recently tested and remotely tested groups within each age group ($p < 0.05$). (^) indicates significant difference between the P25 and P16 age group ($p < 0.05$). (#) indicates significant difference between the P50 age groups and the P18 age groups tested recently ($p < 0.05$). Data are presented as mean $\pm$ SEM.

A.

B.

P16

P50

Recent

Remote

Figure 6. (A) Representative image of the medial prefrontal cortex stained with NeuN outlining the area of the ACC used for cell counts. NeuN protein is found within the nucleus and perinuclear cytoplasm of neurons (Gusel'nikova and Korzhevsky, 2015). It was used to highlight the count region used for the c-Fos count. (B) Representative images of c-Fos levels in the ACC following recent and remote testing for the P16 and 50 age groups.

Figure 7. c-Fos quantification in the ACC. (!!) indicates significant difference between the recently tested and remotely tested groups within each age group ($p < 0.01$). (*) indicates significant difference between the P18 and the P30 and 50 age groups tested remotely ($p < 0.05$). Data are presented as mean $\pm$ SEM.

3.3. Western blotting

3.3.1. GluR1

Quantification of GluR1 levels within the hippocampus is shown in in Figure 8A. A fixed factor ANOVA on GluR1 levels in the hippocampus revealed no main effect of age ($F_{(5, 62)} = 1.894, p = 0.108$), or testing interval ($F_{(1, 62)} = 2.254, p = 0.138$), but did reveal a significant age by testing interval interaction ($F_{(5, 62)} = 3.204, p < 0.05$). Tukey's post-hoc revealed significantly higher levels of GluR1 at P16 compared to P20, P25, and 30 age groups ($p < 0.05$) tested at the remote time point.

Quantification of GluR1 levels within the ACC is shown in in Figure 8C. A fixed factor ANOVA on GluR1 levels in the ACC revealed main effects of age ($F_{(5, 55)} = 4.110, p < 0.01$), testing interval ($F_{(1, 55)} = 7.896, p < 0.01$), and a significant age by testing interval interaction ($F_{(5, 56)} = 2.694, p < 0.05$). Tukey's post-hoc revealed significantly higher levels of GluR1 at P50 compared to P16, 18, 20, and 25 age groups ($p < 0.05$) tested at the recent time point. These results indicate more GluR1 associated with the older ages and more GluR1 in the ACC associated with the remote memory test.

Figure 8. GluR1 expression and representative images in (A-B) the hippocampus and (C-D) the ACC for each age group following recent or remote testing in the open field task. (#) indicates significant differences between P16 and P20, 25, and 30 when tested remotely ($p < 0.05$). (*) indicates significant difference between P50 and P16, 18, 20, and 25 age groups when tested recently ($p < 0.05$). Data are presented as mean $\pm$ SEM.

3.3.2. GluR2

Quantification of GluR2 levels within the hippocampus is shown in Figure 9A. A fixed factor ANOVA on GluR2 levels in the hippocampus revealed a main effect of age ($F_{(5, 62)} = 19.899, p < 0.001$), but no main effect of testing interval ($F_{(1, 62)} = 1.834, p = 0.181$) or age by testing interval interaction ($F_{(5, 62)} = 1.680, p = 0.153$).

Quantification of GluR2 levels within the ACC is shown in Figure 9C. A fixed factor ANOVA on GluR2 levels in the ACC revealed a main effect of age ($F_{(5, 57)} = 4.595, p < 0.01$) and testing interval ($F_{(1, 57)} = 7.322, p < 0.01$), but no age by testing interval interaction ($F_{(5, 57)} = 1.528, p = 0.196$). Similar to GluR1 in the ACC, GluR2 protein levels in the ACC were higher in the older groups and higher in the remote-tested groups.

Figure 9. GluR2 expression and representative images in (A-B) the hippocampus and (C-D) the ACC for each age group following recent or remote testing in the open field task. Data are presented as mean $\pm$ SEM.

4. Discussion

The objective of the current investigation was to assess the transition from juvenile- to adult-like recent and remote memory processing using an open field task at different developmental timepoints (P16, 18, 20, 25, 30, or 50). Rats underwent a single-trial training phase followed by a single-trial test in the same arena either recently (24 hours) or remotely (3 weeks). The difference in distance traveled between testing and training was used as a measure of spatial retention. Reduced exploratory behavior at the time of testing signified that the subject recognized the environment as familiar leading to habitation and less exploratory behavior. Results showed poor performance in P16 to P20 (young cohort) groups at both testing intervals, while the P25 group began to show improved performance akin to that observed at P50. Interestingly, while rats at P30 showed intact spatial memory at the recent testing timepoint, paralleling the performance of the P25 and P50 age groups, their remote testing performance was reminiscent of that seen in the P16-20 groups, i.e. an increase in distance traveled between training and testing. Analyses conducted on the ratio of distance traveled in the center to total distance traveled in the open field revealed no significant difference between training and testing at either 24 hour or 3 week interval across age groups. As such, we do not believe that these data account for the changes in exploration.

There was a gradual improvement in recent spatial memory function from P16 – P20 and by P25, recent memory function matched the level seen in adults (P50). Previous investigations assessing spatial memory during development corroborate these findings, reporting that rats have the capacity to acquire spatial memories by P18, yet cannot retain the information in recent stores until P21 (Rudy et al., 1987; Brown and Whishaw, 2000; Akers and Hamilton, 2007; Wills et al., 2014).

The developmentally-dependent variation in recent spatial memory processing capabilities observed aligns with that of postnatal hippocampal development. The hippocampus undergoes extensive remodeling between P18 and P25, characterized by increased neurogenesis, synaptogenesis, metabolic activity, and complex spine formation (Cotman et al., 1973; Crain et al., 1973; Meibach et al., 1981; Pokorný and Yamamoto, 1981; Schenk, 1985; Holahan et al., 2006, 2007, 2019). Given that spatial memory processing is highly dependent on hippocampal function, it is conceivable that hippocampal maturation is required to support the processing of spatial information in the recent timeframe (Scoville and Milner, 1957; Altman and Bayer, 1975; Squire and Alvarez, 1995; Frankland and Bontempi, 2005; Hartley et al., 2007; Jeneson et al., 2011; Akers et al., 2012). Prior to this period of development, spatial memory acquisition and retrieval is likely relegated to sensory processing in the form of visual, odor, texture, cues which contain general, non-specific information about location in space (Pattwell et al., 2013). Rats trained at P16, 18, and 20 and tested recently (P17-21) had acquired the spatial information prior to or during the initial onset of hippocampal remodeling, and therefore did not possess the hippocampal framework necessary to support the retention of the information. The state of hippocampal maturation by P25 was such that the ability to maintain spatial information for at least 24 hours emerged and was stable into adulthood.

The developmental pattern for remote memory retention was similar for that of recent memory processing. By P25, rats had the capacity to consolidate spatial information into remote stores as indicated by a decrease in exploratory behavior between training (P25) and testing (P46), which paralleled the behavior of the adult group trained on P50 and remotely tested at P71. The younger groups (P16-20) showed impaired performance when tested remotely (P37-41) based on the increase in exploratory behavior from training to testing. The results parallel other

investigations assessing remote memory retention during development. These reports indicate the emergence of remote memory processing capabilities to occur sometime after P20 but before P30 (Campbell and Campbell, 1962; Akers et al., 2012; Guskjolen et al., 2017; Tzakis and Holahan, under review).

Hypotheses outlining the consolidation of memories from recent to remote stores point to hippocampal-cortical connectivity as a means of recent to remote memory transference. These hypotheses posit that the hippocampus integrates information into the cortex through the strengthening of connections between both regions, after which the intracortical connections strengthen through recall and dissociate from the hippocampus (Frankland and Bontempi, 2005). This leads to the eventual independence of the remote memory trace from hippocampal activity (Squire and Alvarez, 1995; Nadel and Moscovitch, 1997; Dudai, 2004; Frankland and Bontempi, 2005; Tzakis and Holahan, under review). The ACC reportedly serves as a repository for remote memory stores given its increase in activity levels during remote memory recall (Weible et al., 2012; Einarsson et al., 2015; Pezze et al., 2017; Tzakis and Holahan, under review). This area shows a developmental trajectory characterized by an increase in cytoarchitectonic and volumetric development, including peak somatic volume and maximal dendritic extension, by P24 (Van Eden et al., 1990; Ferguson and Gao, 2015). The developmental pattern reported for the hippocampus and ACC may provide an explanation for our behavioral data. Improved performance during the remote probe test was first observed in rats trained at P25 which coincides with the period of peak hippocampal and cortical development. Prior to this time (P16-20), rats exhibited poor performance during the probe test indicating that the emergence of remote memory processing requires the late maturation of both the hippocampal and cortical

regions. The strengthening and maturation of connections between both regions begets the capacity to support the transfer of recent spatial memories into remote stores.

Despite the improvements in remote probe test performance observed in the P25 (tested at P46) and P50 (tested at P71) groups, the performance of the P30 group (tested at P51) appeared to revert back to that observed during an earlier, pre-hippocampal/cortical maturation timepoint. Similar declines in performance during this time have been observed in investigations of fear memory. A decrease in fear memory retention in adolescent rats and mice (~P29-33) relative to their pre-adolescent and adult cohorts has been reported (McCallum et al., 2010; Kim et al., 2011; Pattwell et al., 2011, 2012, 2013). The protracted development of the hippocampus and ACC may serve to explain the observed behaviors during this time. The medial prefrontal cortex (mPFC) undergoes volumetric changes throughout development which peak at P27 followed by a reorganization of synaptic connections with a loss of 50% of synapses, resulting in a subsequent decline in gray matter (Huttenlocher, 1984; Mrzljak et al., 1990; Van Eden et al., 1990; Blakemore and Choudhury, 2006; McCallum et al., 2010; Kim et al., 2011). The anterior hippocampus in humans undergoes similar volumetric reductions as a function of age that may be due to differences in synaptic pruning and myelination during adolescence (Gogtay et al., 2006; Pattwell et al., 2013). These volumetric reductions could be associated with substantial cognitive deficits during this time. The pruning of synapses could potentially cause the loss of acquired information, a reduction in hippocampal-cortical connectivity and subsequent impairment of recent to remote memory transfer. Given this, it is conceivable that the initial hippocampal-cortical framework necessary to support remote memory processing is fully functional by P25 but that during the adolescent phase, this network comes “offline” and there is an inability for the transfer of memories from recent to remote stores. Sometime thereafter, these

regions reach full maturity, bringing the networks required to support the formation of recent to remote memories back “online”.

The pattern of c-Fos labeling in the CA1 showed significantly higher levels in the older cohort relative to the younger one during the recent test, which coincided with improved performance during testing in the older group. These results highlight the necessity of CA1 activity for the recall of recent spatial memories and further suggest that the capacity to support such processes comes after the period of hippocampal remodeling (Dillon et al., 2008; Comba et al., 2015; Tzakis et al., 2016; Tzakis and Holahan, under review). As such, the increased CA1 activation and coincident improved performance is likely mediated by the progressive hippocampal development occurring this time and evidently begins to reach adult-like maturity sometime between P21 and P25. ACC activity during the recent testing timepoint was relatively low, as indicated by c-Fos levels, and showed little variation across the different age groups. This was to be expected and coincides with previous research showing little ACC contribution to recent spatial memory processing (Frankland and Bontempi, 2005; Winocur et al., 2010; Nadel and Hardt, 2011). These results outline the dependency of CA1 activity for the expression of recent memory representations and coinciding behavioral output.

Following remote testing, the CA1 region in the older cohort showed decreased activation relative to the younger one despite showing superior remote memory performance. This pattern of CA1 activity aligns with the systems consolidation hypothesis and indicates that by P25, hippocampal development has reached the stage where it can support the transference of recent information to remote stores, rendering the memory trace hippocampus-independent. The increase in hippocampal activity within the younger cohort may be indicative of the processing of novel, recently acquired spatial information, despite being in a familiar environment, which

would explain their poor performance during the remote test. This further suggests that the hippocampus has yet to develop the capacity to transfer information into remote memory stores prior to P25.

Based on the systems consolidation hypothesis, the decreased hippocampal activity observed in the older cohort should coincide with an increase in ACC activity during the remote test. While there was an overall elevation in ACC c-Fos labeling in the remote tested groups at all ages, the younger cohort showed greater ACC activity following remote testing, despite their lack of remote spatial memory. This may speak to the novelty of the behavioral task used in this investigation. In tasks such as the water and radial arm mazes, animals have to integrate spatial information with a goal such as the escape platform or food. As such, increased activity observed in the ACC following a remote probe test in a goal-oriented task may subserve the expression of both the remotely acquired spatial information in conjunction with the goal memory (Kyd and Bilkey, 2003; Hok et al., 2005; Insel and Barnes, 2015). Given that the task used in the current investigation had no discernable goal, ACC involvement during the remote test might only be slightly elevated relative to the recent test, which would represent its contribution to remote memory storage and expression. This is evident in the older, remote-tested cohort whereby ACC activity is only slightly elevated relative to the recent test, but it is less than the younger, remote-tested groups. In the absence of a goal, ACC activity is relegated to the expression of consolidated remote spatial memories alone, and as such any activity observed is solely that which is necessary to express such information. The increased activity within the ACC seen in the younger groups may denote the effortful strain put on the region for remote recall in the absence of any consolidated spatial information acquired during training. This lack of consolidated information manifested as increased exploratory behavior, likely in search for the

goal of the task, in effect “resetting” both regions such that they have to reprocess the spatial information and goal memory, as if the familiar environment were novel again.

Hippocampal GluR1 levels showed a marked decrease after P20, which attenuated at P25 and remained stable into adulthood. This attenuation at P25 coincided with improved recent and remote spatial memory retention. Patterns of hippocampal GluR1 levels undergo developmental flux during the early postnatal timepoint, whereby their levels gradually increase and peak by around P21 (Pellegrini-Giampietro et al., 1992; Pickard et al., 2000; Henley and Wilkinson, 2016). During this time, memory processing is in its infancy, and any memory elicited is generally remedial in form, i.e. sensory-based memories, and recent (~24 hours) (Hayne et al., 1986; Wilson and Sullivan, 1994; Glass et al., 2008; Reger et al., 2009; Pattwell et al., 2013). As such, hippocampal GluR1 likely mediates an immature form of memory processing early in development, forming incongruent, short-lasting memory engrams, and may serve to explain the poor performance observed in the young cohort following either recent or remote testing. The change in GluR1 levels after P25 onward likely denotes this switch and the resulting improvements in performance of the older cohort during both recent and remote testing.

The pattern of GluR1 levels in the ACC showed overall higher levels in the remote-tested groups which was more evident in the P16 – P25 groups. It was by P25 that remote memory function was observed behaviorally. Expression and prolongation of LTP within the ACC has been demonstrated to require the functional recruitment of postsynaptic GluR1, which occurs shortly after LTP induction (Toyoda et al., 2007). The increases in GluR1 levels may outline the degree of synaptic plasticity occurring within the ACC, which evidently increases as a function of age. This increased propensity of GluR1-mediated synaptic plasticity throughout development underscores the developmental synaptic changes necessary for the ACC to begin supporting

remote memory processes, given that GluR1 levels show a change during a time that aligns with the emergence of remote memory behaviors. Information processed prior to this time could not be retained as the ACC likely did not possess the synaptic mechanisms necessary to promote remote memory capabilities.

The pattern of hippocampal GluR2 levels showed an overall attenuation and stabilization by P18, with slight variations across development. While this pattern may not necessarily predict behavioral performance, it does demonstrate the requirement for consistent hippocampal GluR2 levels for the stabilization of the synapse and memory processes. The GluR2 subunit regulates and dictates many of the biophysical properties of the AMPAr, including receptor kinetics and Ca^{+2} permeability, and is expressed at nearly all AMPAr synapses by P14. Furthermore, they regulate homeostatic synaptic scaling, preventing the occurrence of runaway excitation and preserving encoded information within the synapses. This function is especially important for a region such as the hippocampus that shows a particular proclivity for epileptiform discharges in times of hyperexcitability. As such, their pattern of expression may be tightly regulated and remain somewhat consistent so as to preserve the integrity of the synapses at all times. Any variation in levels may parallel increases or decreases in other Ca^{+2} -permeable AMPAr subunits that have the potential to overexcite the synapse if they go unregulated. As previously mentioned, GluR1 levels are elevated until around P20. During this time, the threshold to induce GluR1-mediated LTP is relatively high. The insertion of GluR2 during this time would be required to prevent hyperexcitability. After P20, GluR1 is replaced with GluR3, lowering the threshold for LTP induction, resulting in the need for less GluR2 at the synapse. This may indicate a point of synaptic maturation, whereby less excitation is required to induce LTP and synaptic changes, in turn increasing the synaptic efficiency, thus indirectly influencing the

development of spatial memory processes. The increase in GluR2 levels at later developmental timepoints may depict the protracted development of GluR2, which has previously been reported to continue well into the 20th postnatal week (Sans et al., 2000; Pandey et al., 2015).

The pattern of GluR2 levels within the ACC showed an increase from P25 onward and was markedly higher at remote timepoints. This coincided with increased ACC involvement in remote memory processes and improved performance during remote testing. During this time, an increase in GluR1 was also observed, which likely mediated an increased induction of LTP and resulting synaptic plasticity, facilitating the emergence of remote memory processes. The increase in GluR2 levels was thus likely a response to the increase in GluR1-mediated excitation in the region in order to prevent hyperexcitability. As such, their increased levels indirectly facilitated the emergence of remote memory processing by preserving the encoded information in the synapses. Prior to this, increased excitation would have likely compromised any consolidated spatial information, resulting in the inability to express those memories at a remote timepoint. This would explain the poor performance in the younger groups as their GluR2 levels are evidently lower during training relative to the older groups based on the levels following recent testing. As such, the information that was encoded during training would have likely been subjected to the excitotoxic reduction in synapses and concurrent loss of information. The increase in GluR2 levels from P25 onward preserved the consolidated information acquired during training, allowing it to be expressed during remote testing, resulting in the improved performance in the older groups.

5. Conclusion

In summary, adult-like performance at either recent or remote testing times emerged at P25, which coincided with development of both the hippocampus and ACC. The hippocampal-cortical network required to support the transfer of recent to remote memory may initially be in place by P25 after which such processes are temporarily suspended due to the ongoing development within the hippocampus and ACC, as evident by the poor remote testing performance observed in the P30 group. By P50, the connections may reform and/or strengthen, reinstating the capability to process remote memories. Patterns of c-Fos staining within the CA1 showed decreased levels following a remote test in the P25-50 groups, indicative of hippocampal disengagement during this time. ACC staining in the younger cohort was higher than that in the older cohort during remote testing, which may indicate a “strain” placed on that region to express information that had not been consolidated. By P25, GluR1 and GluR2 levels were at adult-like levels and remained stable, for the most part. This coincides with the emergence of both recent and remote memory processes and concurrent improvement of testing performance. They may contribute to such processes either by directly mediating LTP and synaptic plasticity, or indirectly by regulating activity at the synapse.

References

- Akers KG, Arruda-Carvalho M, Josselyn SA, Frankland PW (2012) Ontogeny of contextual fear memory formation, specificity, and persistence in mice. *Learn Mem* 19:598–604.
- Akers KG, Hamilton DA (2007) Comparison of developmental trajectories for place and cued navigation in the Morris water task. *Dev Psychobiol* 49:553–564.
- Altman J, Bayer S (1975) Postnatal Development of the Hippocampal Dentate Gyrus Under Normal and Experimental Conditions. In: *The Hippocampus*, pp 95–122. Boston, MA: Springer US.
- Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124:319–335.
- Amaral DG, Dent JA (1981) Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions. *J Comp Neurol* 195:51–86.
- Andrásfalvy BK, Magee JC (2004) Changes in AMPA receptor currents following LTP induction on rat CA1 pyramidal neurones. *J Physiol* 559:543–554.
- Bayer SA (1980) Development of the hippocampal region in the rat I. Neurogenesis examined with 3H-thymidine autoradiography. *J Comp Neurol* 190:87–114.
- Blair MG, Nguyen NNQ, Albani SH, L'Etoile MM, Andrawis MM, Owen LM, Oliveira RF, Johnson MW, Purvis DL, Sanders EM, Stoneham ET, Xu H, Dumas TC (2013) Developmental changes in structural and functional properties of hippocampal AMPARs parallels the emergence of deliberative spatial navigation in juvenile rats. *J Neurosci* 33:12218–12228.

- Blakemore SJ, Choudhury S (2006) Development of the adolescent brain: Implications for executive function and social cognition. *J Child Psychol Psychiatry Allied Discip* 47:296–312.
- Bontempi B, Laurent-Demir C, Destrade C, Jaffard R (1999) Time-dependent reorganization of brain circuitry underlying long-term memory storage. *Nature* 400:671–675.
- Bredt DS, Nicoll RA (2003) AMPA receptor trafficking at excitatory synapses. *Neuron* 40:361–379.
- Brown RW, Whishaw IQ (2000) Similarities in the development of place and cue navigation by rats in a swimming pool. *Dev Psychobiol* 37:238–245.
- Campbell BA, Campbell EH (1962) Retention and extinction of learned fear in infant and adult rats. *J Comp Physiol Psychol* 55:1–8.
- Comba R, Gervais N, Mumby D, Holahan M (2015) Emergence of spatial behavioral function and associated mossy fiber connectivity and c-Fos labeling patterns in the hippocampus of rats. *F1000Research* 4.
- Cotman C, Taylor D, Lynch G (1973) Ultrastructural changes in synapses in the dentate gyrus of the rat during development. *Brain Res* 63:205–213.
- Crain B, Cotman C, Taylor D, Lynch G (1973) A quantitative electron microscopic study of synaptogenesis in the dentate gyrus of the rat. *Brain Res* 63:195–204.
- Curlik DM, DiFeo G, Shors TJ (2014) Preparing for adulthood: Thousands upon thousands of new cells are born in the hippocampus during puberty, and most survive with effortful learning. *Front Neurosci* 8.

- Dillon GM, Qu X, Marcus JN, Dodart JC (2008) Excitotoxic lesions restricted to the dorsal CA1 field of the hippocampus impair spatial memory and extinction learning in C57BL/6 mice. *Neurobiol Learn Mem* 90:426–433.
- Dudai Y (2004) The Neurobiology of Consolidations, Or, How Stable is the Engram? *Annu Rev Psychol* 55:51–86.
- Dumas TC (2012) Postnatal alterations in induction threshold and expression magnitude of long-term potentiation and long-term depression at hippocampal synapses. *Hippocampus* 22:188–199.
- Dvorkin A, Benjamini Y, Golani I (2008) Mouse cognition-related behavior in the open-field: emergence of places of attraction. *PLoS Comput Biol* 4:e1000027.
- Einarsson E, Pors J, Nader K (2015) Systems reconsolidation reveals a selective role for the anterior cingulate cortex in generalized contextual fear memory expression. *Neuropsychopharmacology* 40:480–487.
- Ferguson BR, Gao WJ (2015) Development of thalamocortical connections between the mediodorsal thalamus and the prefrontal cortex and its implication in cognition. *Front Hum Neurosci* 8.
- Frankland PW, Bontempi B (2005) The organization of recent and remote memories. *Nat Rev Neurosci* 6:119–130.
- Gaarskjaer FB (1985) The development of the dentate area and the hippocampal mossy fiber projection of the rat. *J Comp Neurol* 241:154–170.
- Gaarskjaer FB (1986) The organization and development of the hippocampal mossy fiber system. *Brain Res* 396:335–357.

- Glass E, Sachse S, Von Suchodoletz W (2008) Development of auditory sensory memory from 2 to 6 years: An MMN study. *J Neural Transm* 115:1221–1229.
- Gogtay N, Nugent TF, Herman DH, Ordonez A, Greenstein D, Hayashi KM, Clasen L, Toga AW, Giedd JN, Rapoport JL, Thompson PM (2006) Dynamic mapping of normal human hippocampal development. *Hippocampus* 16:664–672.
- Gusel'nikova VV, Korzhevsky DE (2015) NeuN as a neuronal nuclear antigen and neuron differentiation marker. *Acta Naturae* 7(2):42-47.
- Guskjolen A, Josselyn SA, Frankland PW (2017) Age-dependent changes in spatial memory retention and flexibility in mice. *Neurobiol Learn Mem* 143:59–66.
- Hartley T, Bird CM, Chan D, Cipolotti L, Husain M, Varga-Khadem F, Burgess N (2007) The hippocampus is required for short-term topographical memory in humans. *Hippocampus* 17:34–38.
- Hayne H, Greco C, Earley L, Griesler P, Rovee-Collier C (1986) Ontogeny of early event memory: II. Encoding and retrieval by 2- and 3-month-olds. *Infant Behav Dev* 9:461–472.
- Henley JM, Wilkinson KA (2016) Synaptic AMPA receptor composition in development, plasticity and disease. *Nat Rev Neurosci* 17:337–350.
- Hok V, Save E, Lenck-Santini PP, Poucet B (2005) Coding for spatial goals in the prelimbic/infralimbic area of the rat frontal cortex. *Proc Natl Acad Sci U S A* 102:4602–4607.
- Holahan MR, Honegger KS, Routtenberg A (2007) Expansion and retraction of hippocampal mossy fibers during postweaning development: Strain-specific effects of NMDA receptor blockade. *Hippocampus* 17:58–67.

- Holahan MR, Rekart JL, Sandoval J, Routtenberg A (2006) Spatial learning induces presynaptic structural remodeling in the hippocampal mossy fiber system of two rat strains. *Hippocampus* 16:560–570.
- Holahan MR, Tzakis N, Oliveira FA (2019) Developmental Aspects of Glucose and Calcium Availability on the Persistence of Memory Function Over the Lifespan. *Front Aging Neurosci* 11.
- Huttenlocher PR (1984) Synapse elimination and plasticity in developing human cerebral cortex. *Am J Ment Defic* 88:488–496.
- Insel N, Barnes CA (2015) Differential activation of fast-spiking and regular-firing neuron populations during movement and reward in the dorsal medial frontal cortex. *Cereb Cortex* 25:2631–2647.
- Jeneson A, Mauldin KN, Hopkins RO, Squire LR (2011) The role of the hippocampus in retaining relational information across short delays: The importance of memory load. *Learn Mem* 18:301–305.
- Jia M, Travaglia A, Pollonini G, Fedele G, Alberini CM (2018) Developmental changes in plasticity, synaptic, glia, and connectivity protein levels in rat medial prefrontal cortex. *Learn Mem* 25:533–543.
- Kessels HW, Malinow R (2009) Synaptic AMPA Receptor Plasticity and Behavior. *Neuron* 61:340–350.
- Kim JH, Li S, Richardson R (2011) Immunohistochemical analyses of long-term extinction of conditioned fear in adolescent rats. *Cereb Cortex* 21:530–538.

- Kroeze Y, Oti M, Van Beusekom E, Cooijmans RHM, Van Bokhoven H, Kolk SM, Homberg JR, Zhou H (2018) Transcriptome analysis identifies multifaceted regulatory mechanisms dictating a genetic switch from neuronal network establishment to maintenance during postnatal prefrontal cortex development. *Cereb Cortex* 28:833–851.
- Kyd RJ, Bilkey DK (2003) Prefrontal cortex lesions modify the spatial properties of hippocampal place cells. *Cereb Cortex* 13:444–451.
- Lipatova O, Campolattaro MM, Toufexis DJ, Mabry EA (2015) Place and response learning in the open-field tower maze. *J Vis Exp* 2015.
- Makino H, Malinow R (2009) AMPA Receptor Incorporation into Synapses during LTP: The Role of Lateral Movement and Exocytosis. *Neuron* 64:381–390.
- Malinow R, Malenka RC (2002) AMPA Receptor Trafficking and Synaptic Plasticity. *Annu Rev Neurosci* 25:103–126.
- Maren S, Tocco G, Standley S, Baudry M, Thompson RF (1993) Postsynaptic factors in the expression of long-term potentiation (LTP): Increased glutamate receptor binding following LTP induction in vivo. *Proc Natl Acad Sci U S A* 90:9654–9658.
- McCallum J, Kim JH, Richardson R (2010) Impaired extinction retention in adolescent rats: Effects of d-cycloserine. *Neuropsychopharmacology* 35:2134–2142.
- Meibach RC, Ross DA, Cox RD, Glick SD (1981) The ontogeny of hippocampal energy metabolism. *Brain Res* 204:431–435.
- Mrzljak L, Uylings HBM, Van Eden GG, Judáš M (1990) Neuronal development in human prefrontal cortex in prenatal and postnatal stages. *Prog Brain Res* 85:185–222.

- Nadel L, Hardt O (2011) Update on memory systems and processes. *Neuropsychopharmacology* 36:251–273.
- Nadel L, Moscovitch M (1997) Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol* 7:217–227.
- Pandey SP, Rai R, Gaur P, Prasad S (2015) Development- and age-related alterations in the expression of AMPA receptor subunit GluR2 and its trafficking proteins in the hippocampus of male mouse brain. *Biogerontology* 16:317–328.
- Pattwell SS, Bath KG, Casey BJ, Ninan I, Lee FS (2011) Selective early-acquired fear memories undergo temporary suppression during adolescence. *Proc Natl Acad Sci U S A* 108:1182–1187.
- Pattwell SS, Duhoux S, Hartley CA, Johnson DC, Jing D, Elliott MD, Ruberry EJ, Powers A, Mehta N, Yang RR, Soliman F, Glatt CE, Casey BJ, Ninan I, Lee FS (2012) Altered fear learning across development in both mouse and human. *Proc Natl Acad Sci U S A* 109:16318–16323.
- Pattwell SS, Lee FS, Casey BJ (2013) Fear learning and memory across adolescent development. *Hormones and behavior special issue: Puberty and adolescence. Horm Behav* 64:380–389.
- Pellegrini-Giampietro DE, Zukin RS, Bennett MVL, Cho S, Pulsinelli WA (1992) Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats. *Proc Natl Acad Sci U S A* 89:10499–10503.
- Penn AC, Zhang CL, Georges F, Royer L, Breillat C, Hosy E, Petersen JD, Humeau Y, Choquet D (2017) Hippocampal LTP and contextual learning require surface diffusion of AMPA receptors. *Nature* 549:384–388.

- Pezze MA, Marshall HJ, Fone KC, Cassaday HJ (2017) Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. *Learn Mem* 24:310–317.
- Pickard L, Noel J, Henley JM, Collingridge GL, Molnar E (2000) Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. *J Neurosci* 20:7922–7931.
- Pinto JGA, Jones DG, Murphy KM (2013) Comparing development of synaptic proteins in rat visual, somatosensory, and frontal cortex. *Front Neural Circuits* 7.
- Pokorný J, Yamamoto T (1981) Postnatal ontogenesis of hippocampal CA1 area in rats. I. Development of dendritic arborisation in pyramidal neurons. *Brain Res Bull* 7:113–120.
- Reger ML, Hovda DA, Giza CC (2009) Ontogeny of rat recognition memory measured by the novel object recognition task. *Dev Psychobiol* 51:672–678.
- Rudy JW, Stadler-Morris S, Albert P (1987) Ontogeny of Spatial Navigation Behaviors in the Rat: Dissociation of “Proximal”-and “Distal”-Cue-Based Behaviors. *Behav Neurosci* 101:62–73.
- Sans N, Petralia RS, Wang YX, Blahos J, Hell JW, Wenthold RJ (2000) A developmental change in NMDA receptor-associated proteins at hippocampal synapses. *J Neurosci* 20:1260–1271.
- Schenk F (1985) Development of place navigation in rats from weaning to puberty. *Behav Neural Biol* 43:69–85.
- Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* 20:11–21.

- Sharma S, Rakoczy S, Brown-Borg H (2010) Assessment of spatial memory in mice. *Life Sci* 87:521–536.
- Shimshek DR, Bus T, Schupp B, Jensen V, Marx V, Layer LE, Köhr G, Sprengel R (2017) Different forms of AMPA receptor mediated LTP and their correlation to the spatial working memory formation. *Front Mol Neurosci* 10.
- Squire LR, Alvarez P (1995) Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol* 5:169–177.
- Teixeira CM, Pomedli SR, Maei HR, Kee N, Frankland PW (2006) Involvement of the anterior cingulate cortex in the expression of remote spatial memory. *J Neurosci* 26:7555–7564.
- Testen A, Ali M, Sexton HG, Hodges S, Dubester K, Reissner KJ, Swartzwelder HS, Risher ML (2019) Region-Specific Differences in Morphometric Features and Synaptic Colocalization of Astrocytes During Development. *Neuroscience* 400:98–109.
- Toyoda H, Wu LJ, Zhao MG, Xu H, Zhuo M (2007) Time-dependent postsynaptic AMPA GluR1 receptor recruitment in the cingulate synaptic potentiation. *Dev Neurobiol* 67:498–509.
- Tzakis N, Bosnic T, Ritchie T, Dixon K, Holahan MR (2016) The effect of AMPA receptor blockade on spatial information acquisition, consolidation and expression in juvenile rats. *Neurobiol Learn Mem* 133:145–156.
- Tzakis N, Holahan MR (under review). Factors that influence the processing of juvenile-acquired spatial memories in rats during hippocampal development. Manuscript submitted for publication.

- Van Eden CG, Kros JM, Uylings HBM (1990) The development of the rat prefrontal cortex: Its size and development of connections with thalamus, spinal cord and other cortical areas. *Prog Brain Res* 85:169–183.
- Vorhees C V., Williams MT (2006) Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* 1:848–858.
- Wartman BC, Gabel J, Holahan MR (2014) Inactivation of the anterior cingulate reveals enhanced reliance on cortical networks for remote spatial memory retrieval after sequential memory processing. *PLoS One* 9.
- Wartman BC, Gervais NJ, Smith C, Comba R, Mumby DG, Holahan MR (2012) Enhanced adolescent learning and hippocampal axonal projections following preadolescent spatial exposure to a water or dry maze. *Brain Res* 1475:37–48.
- Wartman BC, Holahan MR (2014) The impact of multiple memory formation on dendritic complexity in the hippocampus and anterior cingulate cortex assessed at recent and remote time points. *Front Behav Neurosci* 8.
- Weible AP, Rowland DC, Monaghan CK, Wolfgang NT, Kentros CG (2012) Neural correlates of long-term object memory in the mouse anterior cingulate cortex. *J Neurosci* 32:5598–5608.
- Wills TJ, Muessig L, Cacucci F (2014) The development of spatial behaviour and the hippocampal neural representation of space. *Philos Trans R Soc B Biol Sci* 369.
- Wilson DA, Sullivan RM (1994) Neurobiology of associative learning in the neonate: Early olfactory learning. *Behav Neural Biol* 61:1–18.

- Winocur G, Moscovitch M, Bontempi B (2010) Memory formation and long-term retention in humans and animals: Convergence towards a transformation account of hippocampal-neocortical interactions. *Neuropsychologia* 48:2339–2356.
- Zhao C, Teng EM, Summers RG, Ming GL, Gage FH (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci* 26:3–11.

Chapter 5

General Discussion

The aim of the current thesis was to examine the development of spatial memory processing capabilities, and underlying regional and cellular correlates, during a period when the hippocampus has been shown to undergo extensive remodeling. The phenotypic development of memory processing capabilities throughout the remodeling period, from both a recent and remote memory processing perspective, was tracked through the use of spatial tasks. Neural activity profiles, using c-Fos, were examined in the hippocampus and ACC to determine whether recent and remote spatial memories are processed similarly in juveniles and adults. Finally, developmental changes of GluR1 and GluR2 subunits within the hippocampus and ACC were evaluated to determine if their changes coincided with the emergence of spatial memory processing, and if those changes facilitated the transition from juvenile- to adult-like cognitive functions.

Chapter 2 assessed whether juvenile spatial memories acquired during a period of hippocampal remodeling persisted beyond 24 hours and stabilized as a remote memory by assessing their performance on the Morris water maze at either recent or remote intervals and comparing it to adults. Manipulations in training were also used in order to determine if any training factors could facilitate the onset of adult-like memory processing during the juvenile period. Their performance during testing was then correlated to c-Fos activity in both the hippocampus and ACC in order to determine whether remote spatial memories are processed similarly in juveniles and adults. While juveniles were able to process recently-acquired spatial information, they were unable to consolidate that information into remote stores until the later

period of hippocampal remodeling. Spaced-training had no effect on the emergence of remote memory processing capabilities earlier in development. Increased c-Fos activity within the ACC and concurrent decreased activity within the hippocampus coincided with improved performance during remote testing, which was seen either after P26, or earlier in spaced-trained rats. The main outcomes from this chapter outlined that the state of hippocampal development between P18 and 20 appears to be such that it has the capability to support the processing of recently acquired spatial memories, which continues to develop and improve until P24, when those processes reach adult-like levels. The ability to consolidate juvenile-acquired spatial information into remote stores, however, begins to emerge after P26, mediated by the development of both the hippocampus and the strengthening of hippocampal-ACC connectivity.

The distribution of GluR1 and GluR2 protein levels during the period of hippocampal remodeling within the hippocampus and ACC were quantified and compared to adult levels in Chapter 3. GluR1 and GluR2 levels within the hippocampus varied throughout the hippocampal remodeling period, reaching adult levels between P24 and 26, while they showed little variation within the ACC during this time. The main outcomes from this chapter identified hippocampal GluR1 and GluR2 developmental patterns akin to that seen during hippocampal development, gradually increasing over time and reaching adult levels by P24. Given that this timeline coincides with the period of hippocampal remodeling, they likely somehow contribute to this remodeling and facilitate the transition from juvenile- to adult-like cognitive functions. The lack of variability within the ACC suggested a developmental timeline of maturation occurring earlier in postnatal development. As such, the ACC is primed for remote memory function earlier in development and waits for the strengthening of connections with the hippocampus, mediated by

hippocampal remodeling, to allow for the transfer of memory representations into its remote stores.

Chapter 4 was an amalgamation of the previous two chapters, where juvenile rats were trained on the open field task and tested in the same arena at either recent or remote intervals. The difference in distance traveled between training and testing was used as a measure of spatial retention. The use of this novel task was to remedy any potential confounds encountered by the water maze task used in Chapter 2, such as swimming abilities and fatigue. Activity within the hippocampus and ACC was assessed with c-Fos, along with GluR1 and GluR2 protein levels in both regions, following testing. Poor performance was observed at both testing intervals in P16 to 20 groups, while adult-like performance was observed by P25. Performance during the remote test in the P30 group was reminiscent of that seen in the P16-20 groups. Patterns of c-Fos staining within the hippocampus decreased following remote testing in the P25-50 groups, which coincided with an increase in ACC staining relative to their recently-tested counterparts. ACC staining in P16-20 groups was higher, however, than the P25-50 groups. GluR1 and GluR2 levels had reached adult-like levels and remained stable, for the most part by P25. The main outcomes of this chapter aligned with the findings from the previous two chapters, corroborating that the emergence of remote processing capabilities occurs after the period of hippocampal remodeling, ~P25. This is mediated by the strengthening of hippocampal-ACC connectivity, allowing for the transference of information to remote stores within the ACC, and the developmental peak in GluR1 and GluR2 levels by P25, which mediate LTP and regulate synaptic activity. The increased ACC c-Fos staining in the P16-20 likely denoted a strain put on that region to express information that had not been consolidated, while the poor testing

performance in the P30 group may be due to the protracted development of the hippocampus and ACC.

The processing and retention of recently acquired spatial information has been shown to coincide with the onset of hippocampal remodeling; the increase in mossy fiber synaptogenesis and projections to CA3 during this period has been shown to mediate the emergence of such processes (Altman and Das, 1965; Bayer, 1980b; Ramírez-Amaya et al., 1999, 2001; Holahan et al., 2006; Zhang et al., 2009; Curlik et al., 2014; Tzakis et al., 2016). Indeed, dramatic improvements in the water maze task from P19 to 20 onward, where the daily latencies to find the hidden platform began to significantly decrease, were observed with a concurrent increase in synaptogenesis (Keeley et al., 2010). This, and other investigations, point to an overlapping timeline of both hippocampal remodeling and the emergence of recent spatial memory processing, which is evident after P20 (Rauch and Raskin, 1984; Rudy et al., 1987; Wartman et al., 2012; Comba et al., 2015). We further corroborate previous findings by showing that, while the ability to retain recently acquired information began to emerge after P18-19, based on the incremental improvements in pathlength and latency measurements across subsequent water maze training days, it was not until ~P24 that this capability was fully developed. Open field performance furthers this notion by showing that rats were incapable of retaining any recently acquired spatial information for 24 hours until P25.

Performance during training and recent testing on both spatial tasks provides insight into the processing capacity of the hippocampus throughout the remodeling period. Prior to the onset of hippocampal remodeling, the state of hippocampal development is such that it is inefficient at processing incoming spatial information. This is evident from training performance in the pre- and early remodeling age groups; rats first trained in the water maze at P18-22 show incremental

improvements across training measures with each successive training day, yet show no preference for the target platform location when tested 24 hours after their last training day, even after being exposed to the task for 8 trials over 3 days. The latter P22-trained group showed seemingly greater improvements on each successive training day than those seen in the P18 group, with the P22-trained group reaching close to asymptotic performance by the last training day. Rats that began their training day on P24 showed greater improvements during training, reaching asymptotic performance by their last day. From this we can deduce that, even after being exposed to the same task over multiple days, rats undergoing training during the early phase of hippocampal remodeling are incapable of efficiently processing recently-acquired spatial information, compromising the retention of that information. Only during the later period of hippocampal remodeling (~P22-24) has the hippocampus reached the developmental capacity to efficiently process recently-acquired information, possibly through the development of the hippocampus' ability to tune out irrelevant, nonreinforced stimuli (Moore, 1979; O'Keefe et al., 1979; Solomon, 1979; Schmajuk, 1984). Performance in the open field test furthers this point; by P25, the efficiency of the hippocampus is such that it can process and retain recently-acquired spatial information from just a single exposure to the task. Prior to this time, a single exposure is not enough to process and retain the novel spatial information. These results underscore the importance of hippocampal remodeling to the processing and retention of recently acquired spatial information.

Comparing the outcomes of Chapters 2 and 4 highlights some important concepts as it pertains to spatial memory processing throughout development. One general commonality that is apparent in both studies is the protracted development of remote memory capabilities, with its emergence likely occurring sometime between P25 and 30. Based on these results, one can infer

that hippocampal remodeling must be complete, or nearing completion, in order to support the transference of remote memory processes, given that these capabilities did not emerge until late during the remodeling period. Even a hippocampus in the midst of its developmental changes is incapable of supporting such processes, as evident by the lack of remote memories for the rats trained between P22 and 24. Prior to this time, the ACC, or at least the synapses within the ACC, has reached its developmental apex based on the results in Chapters 3 and 4, and is likely “waiting” for the termination of hippocampal remodeling before participating in the strengthening of its connections with the hippocampus. This strengthening would thus lead to the consolidation of memories into remote stores within the ACC. This highlights the impactful role of the hippocampus and the dependence on its development in both recent and remote memory processes.

The consistent behavioral patterns observed across the different age groups and testing intervals between both spatial tasks used in the current thesis validate the use of the open field task as a novel approach to investigating the development of spatial memory. This task was employed with the intent of remedying any potential sensorimotor confounds associated with the use of the water maze task (Vorhees and Williams, 2006; Sharma et al., 2010; Wartman et al., 2012; Comba et al., 2015). Although somewhat novel, variants of the open field task have previously been used to assess spatial cognition (Vazdarjanova and Guzowski, 2004; Dvorkin et al., 2008; Ramirez-Amaya et al., 2013; Lipatova et al., 2015). The use of this task further reaffirms the validity of the behavioral results garnered through the use of the water maze. That is, it confirms that the performance observed during training and testing was indicative of the state of hippocampal development and spatial information processing capacity at that time, and not a result of any extraneous sensorimotor confounds. Future investigations using this task as a

measure of spatial memory capabilities may want to consider such modifications as the addition of a task goal or training trials that span over a few days as opposed to a single exposure, in order to gain further insight into spatial cognitive abilities throughout development.

Another similarity is the regional contribution, or lack thereof, to remote testing performance outcomes. In both studies, an increase in ACC activity and concurrent decrease in CA1 activity relative to recent training manifested as superior performance during remote testing in the older cohorts (training day $\leq$ P25), for the most part. Based on these observations, one can surmise that the initial acquisition and processing of spatial information is dependent on CA1 functioning exclusively for the short-term, after which it is consolidated into remote stores within the ACC, such that its expression is solely reliant on ACC activity, and independent of the hippocampus.

There remains much uncertainty as to the fate of newly acquired spatial information. While hippocampal involvement in the processing of newly acquired information is, for the most part, unanimously agreed upon, the question remains if it relinquishes its role in the processing of that information afterwards, delegating it to the remote stores of the ACC, or if it is constantly involved in the recall of that information. These questions have formulated three main hypotheses in terms of hippocampal involvement: the cognitive map theory (constant hippocampal involvement; O'Keefe and Nadel, 1978), the multiple trace theory (remote hippocampal-neocortical involvement; Nadel and Moscovitch, 1997; Moscovitch et al., 2005, 2006), and the standard consolidation model (complete hippocampal disengagement; Moscovitch et al., 2006).

The aforementioned results are one of the major tenets of the systems consolidation hypothesis and provide credence to the likelihood that information is consolidated in this

manner. Such patterns, though evident in the P20-22 and P22-24 rats, are observed to a lesser extent, which may contribute to the poorer performance observed. Although poorer, their performance trumps that observed in P16-20 rats tested remotely, while eliciting hippocampal and cortical activity that more closely resembles successful systems consolidation relative to the P16-20 counterparts. These observations point to a hippocampal remodeling-mediated progression of remote spatial memory processing capabilities; as remodeling progresses, hippocampal-cortical connectivity strengthens and is more capable of stabilizing a labile memory engram, such that it can be encoded into remote stores. This ability can first be seen early on in hippocampal remodeling, after which it becomes more evident sometime between P24-26, a late period in remodeling when the newly formed connections remain stable into adulthood (Altman and Das, 1965; Bayer, 1980b, 1980a; Holahan et al., 2006; Zhang et al., 2009; Curlik et al., 2014). Previous investigations can corroborate this notion, reporting that rats form remote contextual fear memories by P30, with some indicating that the onset may be as early as around P24 (Campbell and Campbell, 1962; Spear, 1979; Rudy and Morledge, 1994; Raineke et al., 2010; Schiffino et al., 2011). Akers et al. (2012) brought up an interesting concept in their report; it has yet to be determined whether the infantile amnesia observed early on in development reflects a failure of storage or a failure of retrieval. As such, it is possible that the intra-hippocampal and intra-cortical connections are formed early on in development, such that they are capable of supporting the storage of recent and remote memories, however, have not yet fully formed connections between the regions that allow them to successfully consolidate into and/or retrieve from remote repositories. As these hippocampal remodeling-mediated connections develop, the propensity to store and recall remote memories becomes evident through remote behavioral testing.

The synaptic variations that occurred as a result of learning furthers the understanding of the synaptic changes that allow for the development of spatial memory processes. Basal levels of hippocampal GluR1 showed a developmental pattern that aligned with the period of hippocampal remodeling; specifically there is a gradual increase in GluR1 levels from P18-22, coinciding with the onset of the hippocampal remodeling period, followed by a peak at P24 which stabilized into late adolescence, coinciding with the late-phase of hippocampal remodeling. The difference in hippocampal GluR1 levels from P18 to P24 was attenuated after exposure to a spatial learning task. This aligns with the notion that activity-induced LTP increases synaptic GluR1 levels, and corroborates previous findings stating that hippocampal LTP is evident in juveniles as young as P18 (Bekenstein and Lothman, 1991; Lee et al., 2003; Meredith et al., 2003; Ju et al., 2004; Malenka and Bear, 2004). While the magnitude in increase of GluR1 levels could be indicative of increased synaptic efficacy and concurrent behavioral improvements in spatial tasks, it may have had an opposing effect. The surge in GluR1 levels could have resulted in LTP saturation at the synapse, which has been shown to occur in juveniles at this time, and may have led to the observed disruption in spatial learning in younger rats (Barnes et al., 1994; Meredith et al., 2003). Around the same time, there is an increase in GluR2 levels, which may serve as a compensatory mechanism to reduce action potential firing and decrease LTP saturation, given its role in synaptic scaling and LTD (Isaac et al., 2007). Prior to the insertion of GluR2, the processing of spatial information by the hippocampus may be compromised due to the occurrence of saturated synapses, preventing the retention of new memories, and the consolidation of recently acquired information into remote stores. This may explain the behavioral deficits observed during this time.

As the hippocampus progressively develops, GluR1 subunits are replaced by GluR3, which reduce the threshold for postsynaptic potentiation and have been suggested to be an underlying factor in the emergence of adult-like memory processing capabilities (Pellegrini-Giampietro et al., 1992; Dumas, 2012; Blair et al., 2013; Pattwell et al., 2013; Henley and Wilkinson, 2016; McHail et al., 2018). This switch may have been inadvertently observed after P20, whereby GluR1 levels return back to basal levels and remain consistent throughout development. Behaviorally, the emergence of remote memory processes was observed shortly after this time (~P25), in spite of these invariable GluR1 levels. This could likely be attributed to the developmental GluR1-GluR3 switch that occurs around this time. While one may suppose that this downregulation of GluR1 would lead to a decrease in LTP, reports have shown this not be the case, suggesting that other subunits may play a critical role in activity-dependent synaptic plasticity, providing further evidence for the role of GluR3 in mature hippocampal processes (Zamanillo et al., 1999; Malinow, 2003). While GluR1 is downregulated during development, it seemingly continues to play a significant role in memory processing, given the significant spike observed in levels observed in P50 and P71 relative to basal levels. The activity-dependent maintenance and persistence of GluR1 levels is evident at this time, maintaining a constant level of the subunit even after the developmental GluR1-GluR3 switch and subsequent downregulation, thus allowing for its continued involvement in memory processes; perhaps it follows the tenets of the proverbial “use it or lose it”.

While GluR1 and GluR2 have certainly proven to play a large role in the development of memory processes, that credit should also be extended to the more intricate components of the synapse which may arguably be the venerable backbone of memory processing. These, of course, include the calcium-dependent protein kinases, TARPs, synaptic proteins, etc. all of which

interact with AMPAR subunits and function in the trafficking, regulation, and stabilization of AMPAR at the synapse; this function is paramount to the development and prolongation of synaptic plasticity, and resulting memory process.

One synaptic protein that deserves special attention, based on its role in remote memory maintenance is the atypical PKC isoform, protein kinase Mzeta (PKM ζ). This synaptic protein has very recently caught the attention of experimental neurobiologists and cognitive neuroscientists alike for its role in memory processes, specifically remote memory processes. It has been credited for being one of the molecular substrates that sustains remote memory storage (Kwapis and Helmstetter, 2014; Wang et al., 2016). While other protein kinases aim to consolidate memory shortly after learning, PKM ζ maintains the information during the storage stage through the maintenance of synaptic potentiation during late-LTP and remote storage. This has shifted the attention of researchers from the induction of memory to the remote maintenance of memory (Kwapis and Helmstetter, 2014; Wang et al., 2016).

LTP induction increases the synthesis of PKM ζ , which continues to increase during LTP maintenance (Serrano et al., 2005; Sacktor, 2008; Kwapis and Helmstetter, 2014). PKM ζ mediates this late-LTP maintenance by indirectly targeting GluR2 for synaptic potentiation. Specifically, it modifies levels of *N*-ethylmaleimide-sensitive fusion protein (NSF), which interacts with GluR2 as part of a homeostatic mechanism maintaining AMPAR at synapses. This PKM ζ -NSF/GluR2 interaction results in a significant increase in AMPAR trafficking, doubling the number of active AMPAR channels, enhancing synaptic transmission and potentiation, and maintaining late-LTP (Sacktor, 2008, 2012; Yao et al., 2008; Evuarherhe et al., 2014). Further evidence for the importance of this molecule in remote memory processing comes from investigations reporting that PKM ζ blockade in either the hippocampus or the mPFC resulted in

the extinction of remotely-stored memories (Serrano et al., 2008; Hardt et al., 2010; He et al., 2011; Evuarherhe et al., 2014). As it pertains to the current thesis, it is possible that PKM ζ facilitated the maintenance of the consolidated memory once the hippocampal-cortical connections were developed enough to support such processes, given the increase in GluR2 within the ACC during remote testing around the time where remote memory was observed behaviorally. These results may further give credence to the systems consolidation model, given that this variation in GluR2 was seen within the ACC, not the hippocampus, during remote timepoints, i.e. increased maintenance of late-LTP only within the ACC at remote timepoints. Lastly, PKM ζ increases the trafficking of GluR3-containing AMPAR to the synapse which, as previously mentioned is important in the stabilization of the synapse and memory engram (Yao et al., 2008). Future investigations into remote memory processes should focus on the variations in GluR3 and PKM ζ throughout development in order to gain insight into how they mediate, if at all, the emergence of remote memory processing. It would be interesting to see if the upregulation of either in younger animals where remote memory has not been observed behaviorally would facilitate those processes, such that they acquire that ability sooner in development.

Given the developmental timeline during which we conducted our analyses, we would be remiss to not discuss the impact of pubertal status on our cellular and behavioral findings. In males, the onset of puberty has been demarcated sometime around P40 (~P38 and 40), with a peripubertal range defined from ~P38 to P60. This period is marked by a rise in gonadotropin-releasing hormone, and subsequent increases in sex hormones, luteinizing hormone, and follicle-stimulating hormone (Schneider, 2013). Neurodevelopmental consequences of the onset of puberty include increased myelination, increased pruning of synapses and axons, especially

within the ACC, and increased spine density in the CA1, all of which are mediated by the increase in estrogen and testosterone during this time (Kovacs et al., 2003; Brenhouse and Andersen, 2011; Schneider, 2013; Frick et al., 2015; Drzewiecki et al., 2016; Juraska and Willing, 2017). The increase in sex hormones has also been shown to positively modulate memory processes, likely through the aforementioned neurodevelopmental changes (Sandstrom et al., 2006; Leonard and Winsauer, 2011; Spritzer et al., 2011; Hawley et al., 2013; Moradpour et al., 2013; Frick et al., 2015; Yagi and Galea, 2019). Our results seemingly concur with these findings, showing an increase in synaptic events within the hippocampus and ACC, along with an increase in ACC efficiency following pubertal onset. This may likely come as a result of the increased synaptic pruning and myelination, based on the decrease in c-Fos activity relative to the pre-pubescent cohorts, in peripubertal rats. These neurodevelopmental alterations during puberty have also coincided with the emergence of adult-like spatial memory processing.

While it may be tempting to attribute the development of such processes to the onset of puberty, this may not be the case according to Juraska and Willing (2017). They argue the lack of evidence that points to the developmental changes in cognitive behavior being a direct result of puberty itself, and the fact that puberty has been shown to alter learning strategies in certain spatial tasks has made it difficult to elucidate its role. They further cite studies which have shown little to no effect of pubertal status on recent or remote spatial memory (Juraska and Willing, 2017). Indeed, our results follow this notion based on the fact that we observed evidence of adult-like spatial memory processing in rats prior to pubertal onset. Although, pre-pubertal rats trained on a spatial task during the late-phase of hippocampal remodeling and then tested during the peripubertal period showed improved remote memory performance relative to those trained and tested prior to the onset of puberty. From this, we can surmise a two-pronged series of

developmental events that mediate the maturation of spatial memory processing: the first prong involves the completed remodeling of the hippocampal network and the resulting strengthening of the hippocampal-cortical connectivity; this would serve as the catalyst for the development of memory processing. The second prong would involve the onset of puberty, which would function to fine-tune the cellular and morphological underpinnings of cognition, resulting in increased and more stable synapses, allowing them to gain a higher capacity to support memory processing, and increased efficiency of regional functionality.

While these studies were planned with the intent of avoiding any confounds, they do not come without their limitations. For one, the potential lack of effect of spaced training in the first chapter may suggest that the intertrial interval of ~5-8 minutes given for each rat may not have been enough to promote a facilitation of memory retention and/or consolidation, at least not at that stage of hippocampal development. The marginal improvement in testing between massed- and spaced-trained rats at each interval suggests that there may be a potential facilitation of memory processing mediated by spaced-training, but that a longer intertrial interval may be necessary to observe drastic improvements in memory retention. Future directions for such studies should implement an intertrial interval longer than 5-8 minutes in order to see such an effect. It would also be interesting to compare the use of different intertrial intervals over development (i.e. 15 minutes vs. 30 minutes vs. 1 hour) in order to gauge the amount of time necessary to facilitate memory processing, and may provide further insight into the state of hippocampal development during that time; perhaps the intertrial interval necessary to facilitate memory processing would decrease with increased hippocampal development.

While the use of Western blotting in the second study may have provided insight into the levels of GluR1 and GluR2 expression within the hippocampus during development, future

studies may consider investigating the localization of those increased levels on the cell. That is, determining whether that increase is occurring within the cytoplasmic, synaptic, and/or perisynaptic regions. This would provide further insight into the state of synaptic development throughout the hippocampal remodeling period, and how these synaptic changes may mediate the development of memory processing.

Similarly, future studies evaluating GluR1 and GluR2 levels following a spatial memory behavioral task may consider assessing phosphorylated GluR1 and GluR2 levels, in addition to GluR1 and GluR2 levels. Given that learning-induced synaptic plasticity is a result of the phosphorylation of GluR1 and GluR2, any observable improvements in spatial memory throughout development could be attributed to variations in phosphorylated GluR1 and/or GluR2. Further analyzing the phosphorylated GluR1/GluR1 and phosphorylated GluR2/GluR2 ratios would provide further insight into synaptic changes that may mediate the improvement of spatial memory processing throughout development.

Conclusion

The current thesis examined the development of spatial memory processing, and its underlying systems and synaptic correlates, in juveniles during a period of hippocampal development. The initial emergence of recent memory processing coincided with the onset of hippocampal remodeling and continued to be refined as this remodeling progressed. Remote memory processes showed a protracted development, manifesting during late-phase hippocampal remodeling. This prolonged development may have been the result of increased hippocampal development and concurrent capacity to process memories, along with the strengthening of hippocampal-cortical connections that occurred overtime. The pattern of juvenile-acquired spatial memory consolidation that coincided with successful performance during testing trials indicates that the hippocampus is initially responsible for the processing of recently acquired information. After some time, it is stabilized and consolidated into remote stores within the ACC, rendering it independent of hippocampal activity. Both recent and remote memory processing are likely mediated by the development of synaptic components, particularly GluR1 and GluR2. GluR1 increases the propensity for LTP-mediated synaptic plasticity, while GluR2 may have mediatory functions, downscaling the synapse in order to prevent LTP saturation. Future investigations into cognitive development should assess the developmental trajectory of GluR3, given that there is a developmental shift from GluR1 to GluR3 during a time when adult-like memory processing emerges. Further investigations into PKM ζ development, and its contribution to the development of memory processing, would yield insight into the prolongation of LTP, and its effect on the emergence of remote memory storage. This would provide a novel understanding of remote memory processing, approaching the subject from less of a memory induction perspective and more from a memory maintenance perspective.

References

- Abrahams S, Morris RG, Polkey CE, Jarosz JM, Cox TCS, Graves M, Pickering A (1999) Hippocampal involvement in spatial and working memory: A structural MRI analysis of patients with unilateral mesial temporal lobe sclerosis. *Brain Cogn* 41:39–65.
- Aggleton JP, Hunt PR, Rawlins JNP (1986) The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. *Behav Brain Res* 19:133–146.
- Aggleton JP, Neave N, Nagle S, Sahgal A (1995) A comparison of the effects of medial prefrontal, cingulate cortex, and cingulum bundle lesions on tests of spatial memory: Evidence of a double dissociation between frontal and cingulum bundle contributions. *J Neurosci* 15:7270–7281.
- Aguirre GK, D’Esposito M (1999) Topographical disorientation: A synthesis and taxonomy. *Brain* 122:1613–1628.
- Akers KG, Arruda-Carvalho M, Josselyn SA, Frankland PW (2012) Ontogeny of contextual fear memory formation, specificity, and persistence in mice. *Learn Mem* 19:598–604.
- Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124:319–335.
- Amaral DG (1993) Emerging principles of intrinsic hippocampal organization. *Curr Opin Neurobiol* 3:225–229.
- Amaral DG, Dent JA (1981) Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions. *J Comp Neurol* 195:51–86.
- Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: A review of anatomical data. *Neuroscience* 31:571–591.

- Andersen P, Bliss TVP, Skrede KK (1971) Lamellar organization of hippocampal excitatory pathways. *Exp Brain Res* 13:222–238.
- Andersen P, Morris R, Amaral D, Bliss T, O'keefe J (2007) *The Hippocampus Book*. New York, NY: Oxford University Press.
- Angevine JB (1965) Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Exp Neurol Suppl* 2:1–70.
- Astur RS, Taylor LB, Mamelak AN, Philpott L, Sutherland RJ (2002) Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task. *Behav Brain Res* 132:77–84.
- Barnes CA, Jung MW, McNaughton BL, Korol DL, Andreasson K, Worley PF (1994) LTP saturation and spatial learning disruption: Effects of task variables and saturation levels. *J Neurosci* 14:5793–5806.
- Bast T, Da Silva BM, Morris RGM (2005) Distinct contributions of hippocampal NMDA and AMPA receptors to encoding and retrieval of one-trial place memory. *J Neurosci* 25:5845–5856.
- Bayer SA (1980a) Development of the hippocampal region in the rat II. Morphogenesis during embryonic and early postnatal life. *J Comp Neurol* 190:115–134.
- Bayer SA (1980b) Development of the hippocampal region in the rat I. Neurogenesis examined with 3H-thymidine autoradiography. *J Comp Neurol* 190:87–114.
- Bayer SA, Altman J (1974) Hippocampal development in the rat: Cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. *J Comp Neurol* 158:55–79.

- Bekenstein JW, Lothman EW (1991) An in vivo study of the ontogeny of long-term potentiation (LTP) in the CA1 region and in the dentate gyrus of the rat hippocampal formation. *Dev Brain Res* 63:245–251.
- Ben-Ari Y, Represa A (1990) Brief seizure episodes induce long-term potentiation and mossy fibre sprouting in the hippocampus. *Trends Neurosci* 13:312–318.
- Bernabeu R, Cammarota M, Izquierdo I, Medina JH (1997) Involvement of hippocampal AMPA glutamate receptor changes and the cAMP/protein kinase A/CREB-P signalling pathway in memory consolidation of an avoidance task in rats. *Brazilian J Med Biol Res* 30:961–965.
- Bianchin M, Walz R, Ruschel AC, Zanatta MS, Da Silva RC, e Silva MB, Paczko N, Medina JH, Izquierdo I (1993) Memory expression is blocked by the infusion of CNQX into the hippocampus and/or the amygdala up to 20 days after training. *Behav Neural Biol* 59:83–86.
- Blair MG, Nguyen NNQ, Albani SH, L'Etoile MM, Andrawis MM, Owen LM, Oliveira RF, Johnson MW, Purvis DL, Sanders EM, Stoneham ET, Xu H, Dumas TC (2013) Developmental changes in structural and functional properties of hippocampal AMPARs parallels the emergence of deliberative spatial navigation in juvenile rats. *J Neurosci* 33:12218–12228.
- Bolhuis JJ, Stewart CA, Forrest EM (1994) Retrograde amnesia and memory reactivation in rats with ibotenate lesions to the hippocampus or subiculum. *Q J Expir Psychology B* 47:129–150.
- Bontempi B, Laurent-Demir C, Destrade C, Jaffard R (1999) Time-dependent reorganization of brain circuitry underlying long-term memory storage. *Nature* 400:671–675.

- Brandeis R, Brandys Y, Yehuda S (1989) The use of the Morris water maze in the study of memory and learning. *Int J Neurosci* 48:29–69.
- Brasted PJ, Humby T, Dunnett SB, Robbins TW (1997) Unilateral lesions of the dorsal striatum in rats disrupt responding in egocentric space. *J Neurosci* 17:8919–8926.
- Bredewold R, Schiavo JK, van der Hart M, Verreij M, Veenema AH (2015) Dynamic changes in extracellular release of GABA and glutamate in the lateral septum during social play behavior in juvenile rats: Implications for sex-specific regulation of social play behavior. *Neuroscience* 307:117–127.
- Brenhouse HC, Andersen SL (2011) Developmental trajectories during adolescence in males and females: A cross-species understanding of underlying brain changes. *Neurosci Biobehav Rev* 35:1687–1703.
- Burgess N, Maguire EA, O’Keefe J (2002) The human hippocampus and spatial and episodic memory. *Neuron* 35:625–641.
- Burnashev N, Monyer H, Seeburg PH, Sakmann B (1992a) Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron* 8:189–198.
- Burnashev N, Schoepfer R, Monyer H, Ruppersberg JP, Günther W, Seeburg PH, Sakmann B (1992b) Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. *Science* (80-) 257:1415–1419.
- Butters N, Soeldner C, Fedio P (1972) Comparison of parietal and frontal lobe spatial deficits in man: extrapersonal vs personal (egocentric) space. *Percept Mot Skills* 34:27–34.
- Cain DP, Saucier D, Hall J, Hargreaves EL, Boon F (1996) Detailed behavioral analysis of water maze acquisition under APV or CNQX: Contribution of sensorimotor disturbances to drug-induced acquisition deficits. *Behav Neurosci* 110:86–102.

- Cammalleri R, Gangitano M, D'Amelio M, Raieli V, Raimondo D, Camarda R (1996) Transient topographical amnesia and cingulate cortex damage: A case report. *Neuropsychologia* 34:321–326.
- Cammarota M, Izquierdo I, Wolfman C, Levi Destein M, Bernabeu R, Jerusalinsky D, Medina JH (1995) Inhibitory avoidance training induces rapid and selective changes in ^3H AMPA receptor binding in the rat hippocampal formation. *Neurobiol Learn Mem* 64:257–264.
- Campbell BA, Campbell EH (1962) Retention and extinction of learned fear in infant and adult rats. *J Comp Physiol Psychol* 55:1–8.
- Canto CB, Wouterlood FG, Witter MP (2008) What does the anatomical organization of the entorhinal cortex tell us? *Neural Plast* 2008.
- Cenquizca LA, Swanson LW (2007) Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Res Rev* 56:1–26.
- Chapillon P, Roulet P (1996) Use of proximal and distal cues in place navigation by mice changes during ontogeny. *Dev Psychobiol* 29:529–545.
- Chevalyere V, Siegelbaum SA (2010) Strong CA2 pyramidal neuron synapses define a powerful disynaptic cortico-hippocampal loop. *Neuron* 66:560–572.
- Cohen NJ, Ryan J, Hunt C, Romine L, Wszalek T, Nash C (1999) Hippocampal system and declarative (Relational) memory: Summarizing the data from functional neuroimaging studies. *Hippocampus* 9:83–98.
- Collingridge GL, Kehl SJ, McLennan H (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J Physiol* 334:33–46.

- Comba R, Gervais N, Mumby D, Holahan M (2015) Emergence of spatial behavioral function and associated mossy fiber connectivity and c-Fos labeling patterns in the hippocampus of rats. *F1000Research* 4.
- Committeri G, Galati G, Paradis AL, Pizzamiglio L, Berthoz A, LeBihan D (2004) Reference frames for spatial cognition: Different brain areas are involved in viewer-, object-, and landmark-centered judgments about object location. *J Cogn Neurosci* 16:1517–1535.
- Curlik DM, DiFeo G, Shors TJ (2014) Preparing for adulthood: Thousands upon thousands of new cells are born in the hippocampus during puberty, and most survive with effortful learning. *Front Neurosci* 8.
- D’Esposito M, Aguirre GK, Zarahn E, Ballard D, Shin RK, Lease J (1998) Functional MRI studies of spatial and nonspatial working memory. *Cogn Brain Res* 7:1–13.
- D’Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev* 36:60–90.
- Dalley R, Ng L, Guillozet-Bongaarts A (2008) Dentate Gyrus. *Nat Preced*:1–16.
- Debiec J, LeDoux JE, Nader K (2002) Cellular and systems reconsolidation in the hippocampus. *Neuron* 36:527–538.
- DiMattia B V, Kesner RP (1988a) Role of the Posterior Parietal Association Cortex in the Processing of Spatial Event Information. *Behav Neurosci* 102:397–403.
- DiMattia B V, Kesner RP (1988b) Spatial Cognitive Maps: Differential Role of Parietal Cortex and Hippocampal Formation. *Behav Neurosci* 102:471–480.
- Dolorfo CL, Amaral DG (1998) Entorhinal cortex of the rat: Topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. *J Comp Neurol* 398:25–48.

- Drzewiecki CM, Willing J, Juraska JM (2016) Synaptic number changes in the medial prefrontal cortex across adolescence in male and female rats: A role for pubertal onset. *Synapse* 70:361–368.
- Dudai Y (2004) The Neurobiology of Consolidations, Or, How Stable is the Engram? *Annu Rev Psychol* 55:51–86.
- Dudchenko PA (2004) An overview of the tasks used to test working memory in rodents. *Neurosci Biobehav Rev* 28:699–709.
- Dumas TC (2012) Postnatal alterations in induction threshold and expression magnitude of long-term potentiation and long-term depression at hippocampal synapses. *Hippocampus* 22:188–199.
- Dumas TC, Foster TC (1995) Developmental increase in CA3-CA1 presynaptic function in the hippocampal slice. *J Neurophysiol* 73:1821–1828.
- Dvorkin A, Benjamini Y, Golani I (2008) Mouse cognition-related behavior in the open-field: emergence of places of attraction. *PLoS Comput Biol* 4:e1000027.
- Einarsson EÖ, Nader K (2012) Involvement of the anterior cingulate cortex in formation, consolidation, and reconsolidation of recent and remote contextual fear memory. *Learn Mem* 19:449–452.
- Ekstrom AD, Arnold AEGF, Iaria G (2014) A critical review of the allocentric spatial representation and its neural underpinnings: Toward a network-based perspective. *Front Hum Neurosci* 8.
- Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. *Nat Med* 4:1313–1317.

- Evuarherhe O, Barker GRI, Savalli G, Warburton EC, Brown MW (2014) Early memory formation disrupted by atypical PKC inhibitor ZIP in the medial prefrontal cortex but not hippocampus. *Hippocampus* 24:934–942.
- Fanselow MS, Dong HW (2010) Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron* 65:7–19.
- Filliat P, Pernot-Marino I, Baubichon D, Lallement G (1998) Behavioral effects of NBQX, a competitive antagonist of the AMPA receptors. *Pharmacol Biochem Behav* 59:1087–1092.
- Fleischmann A, Hvalby O, Jensen V, Strekalova T, Zacher C, Layer LE, Kvello A, Reschke M, Spanagel R, Sprengel R, Wagner EF, Gass P (2003) Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-fos in the CNS. *J Neurosci* 23:9116–9122.
- Foster JA, Burman MA (2010) Evidence for hippocampus-dependent contextual learning at postnatal day 17 in the rat. *Learn Mem* 17:259–266.
- Frankland PW, Bontempi B (2005) The organization of recent and remote memories. *Nat Rev Neurosci* 6:119–130.
- Frey U, Morris RGM (1997) Synaptic tagging and long-term potentiation. *Nature* 385:533–536.
- Frey U, Morris RGM (1998) Weak before strong: Dissociating synaptic tagging and plasticity-factor accounts of late-LTP. In: *Neuropharmacology*, pp 545–552.
- Frick KM, Kim J, Tuscher JJ, Fortress AM (2015) Sex steroid hormones matter for learning and memory: Estrogenic regulation of hippocampal function in male and female rodents. *Learn Mem* 22:472–493.
- Fricke R, Cowan WM (1977) An autoradiographic study of the development of the entorhinal and commissural afferents to the dentate gyrus of the rat. *J Comp Neurol* 173:231–250.

- Gainey MA, Hurvitz-Wolff JR, Lambo ME, Turrigiano GG (2009) Synaptic scaling requires the GluR2 subunit of the AMPA receptor. *J Neurosci* 29:6479–6489.
- Galati G, Lobel E, Vallar G, Berthoz A, Pizzamiglio L, Bihan D Le (2000) The neural basis of egocentric and allocentric coding of space in humans: A functional magnetic resonance study. *Exp Brain Res* 133:156–164.
- Gallagher M, Burwell R, Burchinal M (2015) Severity of spatial learning impairment in aging: Development of a learning index for performance in the morris water maze. *Behav Neurosci* 129:540–548.
- Gerlai R, Henderson JT, Roder JC, Jia Z (1998) Multiple behavioral anomalies in GluR2 mutant mice exhibiting enhanced LTP. *Behav Brain Res* 95:37–45.
- Granger R, Staubli U, Davis M, Perez Y, Nilsson L, Rogers GA, Lynch G (1993) A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning-dependent task. *Synapse* 15:326–329.
- Greengard P, Jen J, Nairn AC, Stevens CF (1991) Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. *Science* (80-) 253:1135–1138.
- Guskjolen A, Josselyn SA, Frankland PW (2017) Age-dependent changes in spatial memory retention and flexibility in mice. *Neurobiol Learn Mem* 143:59–66.
- Hardt O, Miguez P V., Hastings M, Wong J, Nader K (2010) PKM ζ maintains 1-day- and 6-day-old long-term object location but not object identity memory in dorsal hippocampus. *Hippocampus* 20:691–695.
- Hawley WR, Grissom EM, Martin RC, Halmos MB, Bart CLS, Dohanich GP (2013) Testosterone modulates spatial recognition memory in male rats. *Horm Behav* 63:559–565.

- He YY, Xue YX, Wang JS, Fang Q, Liu JF, Xue LF, Lu L (2011) PKM Maintains drug reward and aversion memory in the basolateral amygdala and extinction memory in the infralimbic cortex. *Neuropsychopharmacology* 36:1972–1981.
- Henley JM, Wilkinson KA (2016) Synaptic AMPA receptor composition in development, plasticity and disease. *Nat Rev Neurosci* 17:337–350.
- Hodges H (1996) Maze procedures: The radial-arm and water maze compared. *Cogn Brain Res* 3:167–181.
- Holahan MR, Honegger KS, Routtenberg A (2007) Expansion and retraction of hippocampal mossy fibers during postweaning development: Strain-specific effects of NMDA receptor blockade. *Hippocampus* 17:58–67.
- Holahan MR, Rekart JL, Sandoval J, Routtenberg A (2006) Spatial learning induces presynaptic structural remodeling in the hippocampal mossy fiber system of two rat strains. *Hippocampus* 16:560–570.
- Hsia AY, Malenka RC, Nicoll RA (1998) Development of excitatory circuitry in the hippocampus. *J Neurophysiol* 79:2013–2024.
- Huganir RL, Nicoll RA (2013) AMPARs and synaptic plasticity: the last 25 years. *Neuron* 80:704–717.
- Insausti R, Amaral DG, Cowan WM (1987) The entorhinal cortex of the monkey: III. Subcortical afferents. *J Comp Neurol* 264:396–408.
- Insel N, Takehara-Nishiuchi K (2013) The cortical structure of consolidated memory: A hypothesis on the role of the cingulate-entorhinal cortical connection. *Neurobiol Learn Mem* 106:343–350.

- Isaac JTR, Ashby M, McBain CJ (2007) The Role of the GluR2 Subunit in AMPA Receptor Function and Synaptic Plasticity. *Neuron* 54:859–871.
- Izquierdo I, Quillfeldt JA, Zanatta MS, Quevedo J, Schaeffer E, Schmitz PK, Medina JH (1997) Sequential Role of Hippocampus and Amygdala, Entorhinal Cortex and Parietal Cortex in Formation and Retrieval of Memory for Inhibitory Avoidance in Rats. *Eur J Neurosci* 9:786–793.
- Jacob FD, Habas PA, Kim K, Corbett-Detig J, Xu D, Studholme C, Glenn OA (2011) Fetal hippocampal development: Analysis by magnetic resonance imaging volumetry. *Pediatr Res* 69:425–429.
- Jerusalinsky D, Ferreira MBC, Walz R, Da Silva RC, Bianchin M, Ruschel AC, Zanatta MS, Medina JH, Izquierdo I (1992) Amnesia by post-training infusion of glutamate receptor antagonists into the amygdala, hippocampus, and entorhinal cortex. *Behav Neural Biol* 58:76–80.
- Jia Z, Lu YM, Agopyan N, Roder J (2001) Gene targeting reveals a role for the glutamate receptors mGluR5 and GluR2 in learning and memory. *Physiol Behav* 73:793–802.
- Jones-Leonard B, McNaughton B, Barnes C (1985) Long-term studies of place field interrelationships in dentate gyrus neurons. *Soc Neurosci Abstr* 11.
- Jonides J, Smith EE, Koeppe RA, Awh E, Minoshima S, Mintun MA (1993) Spatial working memory in humans as revealed by PET. *Nature* 363:623–625.
- Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, Adams SR, Garner CC, Tsien RY, Ellisman MH, Malenka RC (2004) Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci* 7:244–253.

- Jung MW, Wiener SI, McNaughton BL (1994) Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *J Neurosci* 14:7347–7356.
- Juraska JM, Willing J (2017) Pubertal onset as a critical transition for neural development and cognition. *Brain Res* 1654:87–94.
- Kajiwara R, Wouterlood FG, Sah A, Boekel AJ, Baks-Te Bulte LTG, Witter MP (2008) Convergence of entorhinal and CA3 inputs onto pyramidal neurons and interneurons in hippocampal area CA1 - An anatomical study in the rat. *Hippocampus* 18:266–280.
- Keeley RJ, Wartman BC, Häusler AN, Holahan MR (2010) Effect of juvenile pretraining on adolescent structural hippocampal attributes as a substrate for enhanced spatial performance. *Learn Mem* 17:344–354.
- Kesner RP, Farnsworth G, DiMattia B V (1989) Double Dissociation of Egocentric and Allocentric Space Following Medial Prefrontal and Parietal Cortex Lesions in the Rat. *Behav Neurosci* 103:956–961.
- Kesner RP, Farnsworth G, Kametani H (1991) Role of parietal cortex and hippocampus in representing spatial information. *Cereb Cortex* 1:367–373.
- Kessels HW, Malinow R (2009) Synaptic AMPA Receptor Plasticity and Behavior. *Neuron* 61:340–350.
- Kobayashi Y, Amaral DG (2007) Macaque monkey retrosplenial cortex: III. Cortical efferents. *J Comp Neurol* 502:810–833.
- Koganezawa N, Canto CB, Witter MP (2011) Development of functional connectivity from pre- and parasubiculum to medial entorhinal cortex. *Neurosci Res* 71:e176.
- Kolb B, Sutherland RJ, Whishaw IQ (1983) A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats. *Behav Neurosci* 97:13–27.

- Kolleker A, Zhu JJ, Schupp BJ, Qin Y, Mack V, Borchardt T, Köhr G, Malinow R, Seeburg PH, Osten P (2003) Glutamatergic plasticity by synaptic delivery of GluR-Blong- containing AMPA receptors. *Neuron* 40:1199–1212.
- Kovacs EG, MacLusky NJ, Leranth C (2003) Effects of testosterone on hippocampal CA1 spine synaptic density in the male rat are inhibited by fimbria/fornix transection. *Neuroscience* 122:807–810.
- Kwak S, Kawahara Y (2005) Deficient RNA editing of GluR2 and neuronal death in amyotrophic lateral sclerosis. *J Mol Med* 83:110–120.
- Kwapis JL, Helmstetter FJ (2014) Does PKM(zeta) maintain memory? *Brain Res Bull* 105:36–45.
- Lavenex P, Amaral DG (2000) Hippocampal-neocortical interaction: A hierarchy of associativity. *Hippocampus* 10:420–430.
- Lavenex P, Suzuki WA, Amaral DG (2002) Perirhinal and parahippocampal cortices of the macaque monkey: Projections to the neocortex. *J Comp Neurol* 447:394–420.
- Lavenex PB, Amaral DG, Lavenex P (2006) Hippocampal lesion prevents spatial relational learning in adult macaque monkeys. *J Neurosci* 26:4546–4558.
- Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* 112:631–643.
- Leonard ST, Winsauer PJ (2011) The effects of gonadal hormones on learning and memory in male mammals: A review. *Curr Zool* 57:543–558.

- Lerma J, Morales M, Ibarz JM, Somohano F (1994) Rectification Properties and Ca²⁺ Permeability of Glutamate Receptor Channels in Hippocampal Cells. *Eur J Neurosci* 6:1080–1088.
- Liang KC, Hon W, Tyan YM, Liao WL (1994) Involvement of hippocampal NMDA and AMPA receptors in acquisition, formation and retrieval of spatial memory in the Morris water maze. *Chin J Physiol* 37:201–212.
- Lipatova O, Campolattaro MM, Toufexis DJ, Mabry EA (2015) Place and response learning in the open-field tower maze. *J Vis Exp* 2015.
- Maccaferri G (2011) Modulation of hippocampal stratum lacunosum-moleculare microcircuits. *J Physiol* 589:1885–1891.
- Maei HR, Zaslavsky K, Teixeira CM, Frankland PW (2009) What is the most sensitive measure of water maze probe test performance? *Front Integr Neurosci* 3.
- Maguire EA, Nannery R, Spiers HJ (2006) Navigation around London by a taxi driver with bilateral hippocampal lesions. *Brain* 129:2894–2907.
- Malenka RC, Bear MF (2004) LTP and LTD: An embarrassment of riches. *Neuron* 44:5–21.
- Malinow R (2003) AMPA receptor trafficking and long-term potentiation. *Philos Trans R Soc B Biol Sci* 358:707–714.
- Martin SJ, Grimwood PD, Morris RGM (2000) Synaptic Plasticity and Memory: An Evaluation of the Hypothesis. *Annu Rev Neurosci* 23:649–711.
- Martin SJ, Morris RGM (2002) New life in an old idea: The synaptic plasticity and memory hypothesis revisited. *Hippocampus* 12:609–636.

- McCarthy G, Blamire AM, Puce A, Nobre AC, Bloch G, Hyder F, Goldman-Rakic P, Shulman RG (1994) Functional magnetic resonance imaging of human prefrontal cortex activation during a spatial working memory task. *Proc Natl Acad Sci U S A* 91:8690–8694.
- McCarthy G, Puce A, Constable RT, Krystal JH, Gore JC, Goldman-Rakic P (1996) Activation of human prefrontal cortex during spatial and nonspatial working memory tasks measured by functional MRI. *Cereb Cortex* 6:600–611.
- McGlade-Mcculloh E, Yamamoto H, Tan SE, Brickey DA, Soderling TR (1993) Phosphorylation and regulation of glutamate receptors by calcium/calmodulin-dependent protein kinase II. *Nature* 362:640–642.
- McHail DG, Valibeigi N, Dumas TC (2018) Barnes maze for juvenile rats delineates the emergence of spatial navigation ability. *Learn Mem* 25:138–146.
- McNaughton BL, Morris RGM (1987) Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends Neurosci* 10:408–415.
- Mednick SC, Cai DJ, Shuman T, Anagnostaras S, Wixted JT (2011) An opportunistic theory of cellular and systems consolidation. *Trends Neurosci* 34:504–514.
- Meeter M, Murre JMJ (2004) Consolidation of long-term memory: Evidence and alternatives. *Psychol Bull* 130:843–857.
- Mehta MA (2014) Spatial memory in humans. In: *Encyclopedia of psychopharmacology* (Stolerman I, ed), pp 1262–1266. Berlin: Springer-Verlag.
- Meredith RM, Floyer-Lea AM, Paulsen O (2003) Maturation of Long-Term Potentiation Induction Rules in Rodent Hippocampus: Role of GABAergic Inhibition. *J Neurosci* 23:11142–11146.

- Micheau J, Riedel G, Roloff EVL, Inglis J, Morris RGM (2004) Reversible hippocampal inactivation partially dissociates how and where to search in the water maze. *Behav Neurosci* 118:1022–1032.
- Monaghan DT, Bridges RJ, Cotman CW (1989) The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu Rev Pharmacol Toxicol* 29:365–402.
- Moore JW (1979) Information processing in space-time by the hippocampus. *Physiol Psychol* 7:224–232.
- Moradpour F, Naghdi N, Fathollahi Y, Javan M, Choopani S, Gharaylou Z (2013) Pre-pubertal castration improves spatial learning during mid-adolescence in rats. *Prog Neuro-Psychopharmacology Biol Psychiatry* 46:105–112.
- Morris RG, Davis S, Butcher SP (1990) Hippocampal synaptic plasticity and NMDA receptors: a role in information storage? *Philos Trans R Soc Lond B Biol Sci* 329:187–204.
- Morris RGM (1981) Spatial localization does not require the presence of local cues. *Learn Motiv* 12:239–260.
- Morris RGM, Garrud P, Rawlins JNP, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. *Nature* 297:681–683.
- Morris RGM, Halliwell RF, Bowery N (1989) Synaptic plasticity and learning II: Do different kinds of plasticity underlie different kinds of learning? *Neuropsychologia* 27:41–59.
- Moscovitch M, Nadel L, Winocur G, Gilboa A, Rosenbaum RS (2006) The cognitive neuroscience of remote episodic, semantic and spatial memory. *Curr Opin Neurobiol* 16:179–190.

- Moscovitch M, Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C, McAndrews MP, Levine B, Black S, Winocur G, Nadel L (2005) Functional neuroanatomy of remote episodic, semantic and spatial memory: A unified account based on multiple trace theory. *J Anat* 207:35–66.
- Moser E, Moser MB, Andersen P (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J Neurosci* 13:3916–3925.
- Moser MB, Moser EI (1998) Functional differentiation in the hippocampus. *Hippocampus* 8:608–619.
- Moser MB, Moser EI, Forrest E, Andersen P, Morris RGM (1995) Spatial learning with a minislab in the dorsal hippocampus. *Proc Natl Acad Sci U S A* 92:9697–9701.
- Muir GM, Bilkey DK (2001) Instability in the place field location of hippocampal place cells after lesions centered on the perirhinal cortex. *J Neurosci* 21:4016–4025.
- Nadel L (1991) The hippocampus and space revisited. *Hippocampus* 1:221–229.
- Nadel L, MacDonald L (1980) Hippocampus: cognitive map or working memory? *Behav Neural Biol* 29:405–409.
- Nadel L, Moscovitch M (1997) Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol* 7:217–227.
- Nakazawa K, McHugh TJ, Wilson MA, Tonegawa S (2004) NMDA receptors, place cells and hippocampal spatial memory. *Nat Rev Neurosci* 5:361–372.
- Neves G, Cooke SF, Bliss TVP (2008) Synaptic plasticity, memory and the hippocampus: A neural network approach to causality. *Nat Rev Neurosci* 9:65–75.

- Norris CM, Foster TC (1999) MK-801 improves retention in aged rats: Implications for altered neural plasticity in age-related memory deficits. *Neurobiol Learn Mem* 71:194–206.
- O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res* 34:171–175.
- O'Keefe J, Nadel L (1978) *The hippocampus as a cognitive map*. Oxford: Oxford University Press.
- O'Keefe J, Nadel L, Keightley S, Kill D (1975) Fornix lesions selectively abolish place learning in the rat. *Exp Neurol* 48:152–166.
- O'Keefe J, Nadel L, Willner J (1979) Tuning out irrelevancy? Comments on Solomon's temporal mapping view of the hippocampus. *Psychol Bull* 86:1280–1289.
- O'Keefe J, Speakman A (1987) Single unit activity in the rat hippocampus during a spatial memory task. *Exp Brain Res* 68:1–27.
- Olton DS, Samuelson RJ (1976) Remembrance of places passed: Spatial memory in rats. *J Exp Psychol Anim Behav Process* 2:97–116.
- Owen AM, Evans AC, Petrides M (1996) Evidence for a Two-Stage Model of Spatial Working Memory Processing within the Lateral Frontal Cortex: A Positron Emission Tomography Study. *Cereb Cortex* 6:31–38.
- Owen AM, Stern CE, Look RB, Tracey I, Rosen BR, Petrides M (1998) Functional organization of spatial and nonspatial working memory processing within the human lateral frontal cortex. *Proc Natl Acad Sci U S A* 95:7721–7726.
- Packard MG, Teather LA (1997a) Double dissociation of hippocampal and dorsal-striatal memory systems by posttraining intracerebral injections of 2-amino-5-phosphonopentanoic acid. *Behav Neurosci* 111:543–551.

- Packard MG, Teather LA (1997b) Intra-hippocampal estradiol infusion enhances memory in ovariectomized rats. *Neuroreport* 8:3009–3013.
- Pattwell SS, Lee FS, Casey BJ (2013) Fear learning and memory across adolescent development. Hormones and behavior special issue: Puberty and adolescence. *Horm Behav* 64:380–389.
- Pellegrini-Giampietro DE, Zukin RS, Bennett MVL, Cho S, Pulsinelli WA (1992) Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats. *Proc Natl Acad Sci U S A* 89:10499–10503.
- Petrides M (1994) Frontal lobes and working memory: evidence from investigations of the effects of cortical excisions in nonhuman primates. In: *Handbook of neuropsychology* (Boller F, Grafman J, eds), pp 59–82. Amsterdam: Elsevier Ltd.
- Pitkänen A, Pikkarainen M, Nurminen N, Ylinen A (2000) Reciprocal Connections between the Amygdala and the Hippocampal Formation, Perirhinal Cortex, and Postrhinal Cortex in Rat: A Review. *Ann N Y Acad Sci* 911:369–391.
- Pohl W (1973) Dissociation of spatial discrimination deficits following frontal and parietal lesions in monkeys. *J Comp Physiol Psychol* 82:227–239.
- Pokorný J, Yamamoto T (1981) Postnatal ontogenesis of hippocampal CA1 area in rats. I. Development of dendritic arborisation in pyramidal neurons. *Brain Res Bull* 7:113–120.
- Pothuizen HHJ, Zhang WN, Jongen-Rêlo AL, Feldon J, Yee BK (2004) Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: A within-subject, within-task comparison of reference and working spatial memory. *Eur J Neurosci* 19:705–712.

- Ragozzino ME, Adams S, Kesner RP (1998) Differential involvement of the dorsal anterior cingulate and prelimbic- infralimbic areas of the rodent prefrontal cortex in spatial working memory. *Behav Neurosci* 112:293–303.
- Raineki C, Holman PJ, Debiec J, Bugg M, Beasley A, Sullivan RM (2010) Functional emergence of the hippocampus in context fear learning in infant rats. *Hippocampus* 20:1037–1046.
- Ramírez-Amaya V, Angulo-Perkins A, Chawla MK, Barnes CA, Rosi S (2013) Sustained transcription of the immediate early gene *arc* in the dentate gyrus after spatial exploration. *J Neurosci* 33:1631–1639.
- Ramírez-Amaya V, Balderas I, Sandoval J, Escobar ML, Bermúdez-Rattoni F (2001) Spatial long-term memory is related to mossy fiber synaptogenesis. *J Neurosci* 21:7340–7348.
- Ramírez-Amaya V, Escobar ML, Chao V, Bermúdez-Rattoni F (1999) Synaptogenesis of mossy fibers induced by spatial water maze overtraining. *Hippocampus* 9:631–636.
- Rauch SL, Raskin LA (1984) Cholinergic mediation of spatial memory in the preweanling rat: Application of the radial arm maze paradigm. *Behav Neurosci* 98:35–43.
- Ray S, Brecht M (2016) Structural development and dorsoventral maturation of the medial entorhinal cortex. *Elife* 5:e13343.
- Reisel D, Bannerman DM, Schmitt WB, Deacon RMJ, Flint J, Borchardt T, Seeburg PH, Rawlins JNP (2002) Spatial memory dissociations in mice lacking GluR1. *Nat Neurosci* 5:868–873.
- Restivo L, Vetere G, Bontempi B, Ammassari-Teule M (2009) The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. *J Neurosci* 29:8206–8214.

- Riedel G, Micheau J (2001) Function of the hippocampus in memory formation: Desperately seeking resolution. *Prog Neuro-Psychopharmacology Biol Psychiatry* 25:835–853.
- Riedel G, Micheau J, Lam AGM, Roloff EVL, Martin SJ, Bridge H, De Hoz L, Poeschel B, McCulloch J, Morris RGM (1999) Reversible neural inactivation reveals hippocampal participation in several memory processes. *Nat Neurosci* 2:898–905.
- Riedel G, Platt B, Micheau J (2003) Glutamate receptor function in learning and memory. *Behav Brain Res* 140:1–47.
- Rockland KS, Van Hoesen GW (1999) Some temporal and parietal cortical connections converge in CA1 of the primate hippocampus. *Cereb Cortex* 9:232–237.
- Rose FC, Symonds CP (1960) Persistent memory defect following encephalitis. *Brain* 83:195–212.
- Rosenbaum RS, Gao F, Richards B, Black SE, Moscovitch M (2005) “Where to?” Remote memory for spatial relations and landmark identity in former taxi drivers with Alzheimer’s disease and encephalitis. *J Cogn Neurosci* 17:446–462.
- Rosenbaum RS, Priselac S, Köhler S, Black SE, Gao F, Nadel L, Moscovitch M (2000) Remote spatial memory in an amnesic person with extensive bilateral hippocampal lesions. *Nat Neurosci* 3:1044–1048.
- Roullet P, Lassalle JM, Jegat R (1993) A study of behavioral and sensorial bases of radial maze learning in mice. *Behav Neural Biol* 59:173–179.
- Rudy JW, Morledge P (1994) Ontogeny of contextual fear conditioning in rats: Implications for consolidation, infantile amnesia, and hippocampal system function. *Behav Neurosci* 108:227–234.

- Rudy JW, Stadler-Morris S, Albert P (1987) Ontogeny of Spatial Navigation Behaviors in the Rat: Dissociation of “Proximal”-and “Distal”-Cue-Based Behaviors. *Behav Neurosci* 101:62–73.
- Rudy JW, Sutherland RJ (2008) Is it systems or cellular consolidation? Time will tell. An alternative interpretation of the Morris group’s recent science paper. *Neurobiol Learn Mem* 89:366–369.
- Sacktor TC (2008) Chapter 2 PKM ζ , LTP maintenance, and the dynamic molecular biology of memory storage. *Prog Brain Res* 169:27–40.
- Sacktor TC (2012) Memory maintenance by PKM an evolutionary perspective. *Mol Brain* 5.
- Sandler R, Smith AD (1991) Coexistence of GABA and glutamate in mossy fiber terminals of the primate hippocampus: An ultrastructural study. *J Comp Neurol* 303:177–192.
- Sandstrom NJ, Kim JH, Wasserman MA (2006) Testosterone modulates performance on a spatial working memory task in male rats. *Horm Behav* 50:18–26.
- Saunders RC, Rosene DL, Van Hoesen GW (1988) Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. Reciprocal and non-reciprocal connections. *J Comp Neurol* 271:185–207.
- Save E, Moghaddam M (1996) Effects of lesions of the associative parietal cortex on the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat. *Behav Neurosci* 110:74–85.
- Save E, Poucet B, Foreman N, Buhot MC (1992) Object Exploration and Reactions to Spatial and Nonspatial Changes in Hooded Rats Following Damage to Parietal Cortex or Hippocampal Formation. *Behav Neurosci* 106:447–456.

- Schiffino FL, Murawski NJ, Rosen JB, Stanton ME (2011) Ontogeny and neural substrates of the context preexposure facilitation effect. *Neurobiol Learn Mem* 95:190–198.
- Schmajuk NA (1984) Psychological theories of hippocampal function. *Physiol Psychol* 12:166–183.
- Schneider M (2013) Adolescence as a vulnerable period to alter rodent behavior. *Cell Tissue Res* 354:99–106.
- Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* 20:11–21.
- Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol* 106–107:1–16.
- Serrano P, Friedman EL, Kenney J, Taubenfeld SM, Zimmerman JM, Hanna J, Alberini C, Kelley AE, Maren S, Rudy JW, Yin JCP, Sacktor TC, Fenton AA (2008) PKM ζ maintains spatial, instrumental, and classically conditioned long-term memories Lu B, ed. *PLoS Biol* 6:2698–2706.
- Serrano P, Yao Y, Sacktor TC (2005) Persistent phosphorylation by protein kinase M ζ maintains late-phase long-term potentiation. *J Neurosci* 25:1979–1984.
- Shapiro ML, Caramanos Z (1990) NMDA antagonist MK-801 impairs acquisition but not performance of spatial working and reference memory. *Psychobiology* 18:231–243.
- Sharma S, Rakoczy S, Brown-Borg H (2010) Assessment of spatial memory in mice. *Life Sci* 87:521–536.
- Siddoway B, Hou H, Xia H (2014) Molecular mechanisms of homeostatic synaptic downscaling. *Neuropharmacology* 78:38–44.

- Smith CN, Squire LR (2009) Medial temporal lobe activity during retrieval of semantic memory is related to the age of the memory. *J Neurosci* 29:930–938.
- Snyder DC, Coltman BW, Muneoka K, Ide CF (1991) Mapping the early development of projections from the entorhinal cortex in the embryonic mouse using prenatal surgery techniques. *J Neurobiol* 22:897–906.
- Solomon PR (1979) Temporal versus spatial information processing theories of hippocampal function. *Psychol Bull* 86:1272–1279.
- Spear NE (1979) Memory storage factors leading to infantile amnesia. *Psychol Learn Motiv - Adv Res Theory* 13:91–154.
- Spiers HJ, Maguire EA (2007) The neuroscience of remote spatial memory: A tale of two cities. *Neuroscience* 149:7–27.
- Spritzer MD, Daviau ED, Coneeny MK, Engelman SM, Prince WT, Rodriguez-Wisdom KN (2011) Effects of testosterone on spatial learning and memory in adult male rats. *Horm Behav* 59:484–496.
- Stepan J, Dine J, Eder M (2015) Functional optical probing of the hippocampal trisynaptic circuit in vitro: Network dynamics, filter properties, and polysynaptic induction of CA1 LTP. *Front Neurosci* 9.
- Stringer JL, Greenfield LJ, Hackett JT, Guyenet PG (1983) Blockade of long-term potentiation by phencyclidine and σ opiates in the hippocampus in vivo and in vitro. *Brain Res* 280:127–138.
- Sutherland RJ, Kolb B, Whishaw IQ (1982) Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rat. *Neurosci Lett* 31:271–276.

- Suzuki WA, Amaral DG (2004) Functional neuroanatomy of the medial temporal lobe memory system. *Cortex* 40:220–222.
- Swanson LW, Cowan WM (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *J Comp Neurol* 172:49–84.
- Szapiro G, Izquierdo LA, Alonso M, Barros D, Paratcha G, Ardenghi P, Pereira P, Medina JH, Izquierdo I (2000) Participation of hippocampal metabotropic glutamate receptors, protein kinase A and mitogen-activated protein kinases in memory retrieval. *Neuroscience* 99:1–5.
- Takashima A, Petersson KM, Rutter F, Tendolkar I, Jensen O, Zwartz MJ, McNaughton BL, Fernández G (2006) Declarative memory consolidation in humans: A prospective functional magnetic resonance imaging study. *Proc Natl Acad Sci U S A* 103:756–761.
- Takehara-Nishiuchi K (2014) Entorhinal cortex and consolidated memory. *Neurosci Res* 84:27–33.
- Tanaka H, Grooms SY, Bennett MVL, Zukin RS (2000) The AMPAR subunit GluR2: Still front and center-stage. *Brain Res* 886:190–207.
- Teixeira CM, Pomedli SR, Maei HR, Kee N, Frankland PW (2006) Involvement of the anterior cingulate cortex in the expression of remote spatial memory. *J Neurosci* 26:7555–7564.
- Teng E, Squire LR (1999) Memory for places learned long ago is intact after hippocampal damage. *Nature* 400:675–677.
- Tocco G, Annala AJ, Baudry M, Thompson RF (1992) Learning of a hippocampal-dependent conditioning task changes the binding properties of AMPA receptors in rabbit hippocampus. *Behav Neural Biol* 58:222–231.

- Tocco G, Devgan KK, Hauge SA, Weiss C, Baudry M, Thompson RF (1991) Classical conditioning selectively increases AMPA receptor binding in rabbit hippocampus. *Brain Res* 559:331–336.
- Tolman EC (1948) Cognitive maps in rats and men. *Psychol Rev* 55:189–208.
- Treves A, Tashiro A, Witter ME, Moser EI (2008) What is the mammalian dentate gyrus good for? *Neuroscience* 154:1155–1172.
- Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391:892–896.
- Tzakis N, Bosnic T, Ritchie T, Dixon K, Holahan MR (2016) The effect of AMPA receptor blockade on spatial information acquisition, consolidation and expression in juvenile rats. *Neurobiol Learn Mem* 133:145–156.
- Tzakis N, Holahan MR (2016) AMPA receptor subunit contribution to hippocampal-mediated spatial memory. In: *Spatial, long- and short-term memory*, pp 31–46.
- Vallar G, Lobel E, Galati G, Berthoz A, Pizzamiglio L, Le Bihan D (1999) A fronto-parietal system for computing the egocentric spatial frame of reference in humans. *Exp Brain Res* 124:281–286.
- van Groen T, Miettinen P, Kadish I (2003) The entorhinal cortex of the mouse: Organization of the projection to the hippocampal formation. *Hippocampus* 13:133–149.
- Van Strien NM, Cappaert NLM, Witter MP (2009) The anatomy of memory: An interactive overview of the parahippocampal- hippocampal network. *Nat Rev Neurosci* 10:272–282.
- Vazdarjanova A, Guzowski JF (2004) Differences in hippocampal neuronal population responses to modifications of an environmental context: Evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *J Neurosci* 24:6489–6496.

- Volpe BT, Hirst W (1983) The Characterization of an Amnesic Syndrome Following Hypoxic Ischemic Injury. *Arch Neurol* 40:436–440.
- Vorhees C V., Williams MT (2006) Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* 1:848–858.
- Vorhees C V, Williams MT (2014) Assessing spatial learning and memory in rodents. *ILAR J* 55:310–332.
- Wang S-H, Morris RGM (2010) Hippocampal-Neocortical Interactions in Memory Formation, Consolidation, and Reconsolidation. *Annu Rev Psychol* 61:49–79.
- Wang S, Sheng T, Ren S, Tian T, Lu W (2016) Distinct Roles of PKC α / λ and PKM ζ in the Initiation and Maintenance of Hippocampal Long-Term Potentiation and Memory. *Cell Rep* 16:1954–1961.
- Wartman BC, Gabel J, Holahan MR (2014) Inactivation of the anterior cingulate reveals enhanced reliance on cortical networks for remote spatial memory retrieval after sequential memory processing. *PLoS One* 9.
- Wartman BC, Gervais NJ, Smith C, Comba R, Mumby DG, Holahan MR (2012) Enhanced adolescent learning and hippocampal axonal projections following preadolescent spatial exposure to a water or dry maze. *Brain Res* 1475:37–48.
- Wartman BC, Holahan MR (2013) The use of sequential hippocampal-dependent and -non-dependent tasks to study the activation profile of the anterior cingulate cortex during recent and remote memory tests. *Neurobiol Learn Mem* 106:334–342.
- Wartman BC, Holahan MR (2014) The impact of multiple memory formation on dendritic complexity in the hippocampus and anterior cingulate cortex assessed at recent and remote time points. *Front Behav Neurosci* 8.

- Wellman BJ, Rockland KS (1997) Divergent cortical connections to entorhinal cortex from area TF in the Macaque. *J Comp Neurol* 389:361–376.
- Wible CG (2013) Hippocampal physiology, structure and function and the neuroscience of schizophrenia: A unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms. *Behav Sci (Basel)* 3:298–315.
- Witter MP (2007) Intrinsic and extrinsic wiring of CA3: Indications for connectional heterogeneity. *Learn Mem* 14:705–713.
- Witter MP, Amaral DG (1991) Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. *J Comp Neurol* 307:437–459.
- Witter MP, Wouterlood FG, Naber PA, Van Haeften T (2006) Anatomical Organization of the Parahippocampal-Hippocampal Network. *Ann N Y Acad Sci* 911:1–24.
- Wright A, Vissel B (2012) The essential role of AMPA receptor GluA2 subunit RNA editing in the normal and diseased brain. *Front Mol Neurosci* 5.
- Yagi S, Galea LAM (2019) Sex differences in hippocampal cognition and neurogenesis. *Neuropsychopharmacology* 44:200–213.
- Yao Y, Kelly MT, Sajikumar S, Serrano P, Tian D, Bergold PJ, Frey JU, Sacktor TC (2008) PKM ζ maintains late long-term potentiation by N-ethylmaleimide- sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. *J Neurosci* 28:7820–7827.
- Yoo DY, Yoo K-Y, Park JH, Choi JW, Kim W, Hwang IK, Won M-H (2011) Detailed Differentiation of Calbindin D-28k-Immunoreactive Cells in the Dentate Gyrus in C57BL/6 Mice at Early Postnatal Stages. *Lab Anim Res* 27:153.
- Zaehle T, Jordan K, Wüstenberg T, Baudewig J, Dechent P, Mast FW (2007) The neural basis of the egocentric and allocentric spatial frame of reference. *Brain Res* 1137:92–103.

- Zamanillo D, Sprengel R, Hvalby Ø, Jensen V, Burnashev N, Rozov A, Kaiser KMM, Köster HJ, Borchardt T, Worley P, Lübke J, Frotscher M, Kelly PH, Sommer B, Andersen P, Seeburg PH, Sakmann B (1999) Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science* (80-) 284:1805–1811.
- Zhang L, Blomgren K, Kuhn HG, Cooper-Kuhn CM (2009) Effects of postnatal thyroid hormone deficiency on neurogenesis in the juvenile and adult rat. *Neurobiol Dis* 34:366–374.
- Zola-Morgan S, Squire LR, Amaral DG (1986) Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *J Neurosci* 6:2950–2967.

Supplemental Data

Supplemental Figure 1. Western blot showing target protein for (A) GluR1 and (B) GluR2.

Supplemental Figure 2. Comparison of performance in the open field task between males and females for each age group. No significant differences observed between males and females at P16 ($p = 0.980$), P18 ($p = 0.916$), P20 ($p = 0.092$), or P25 ($p = 0.730$). Given that no apparent significant differences in performance were noted between males and females, the data from both sexes were collapsed across each age group.