The effects of REM sleep deprivation on spatial and reversal learning

By

Christine Mairéad Quinn Walsh

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Neuroscience)
in The University of Michigan
2010

Doctoral Committee:

Associate Professor Gina R. Poe, Chair
Professor Stephen A. Maren
Professor Mark R. Opp
Assistant Professor Victoria Booth
Assistant Professor Geoffrey G. Murphy

Dedication

To My Mom

Acknowledgements

The methodology for reaching my dissertation; a reversed chronological description of two graduate career experiments:

Experiment 2

I would like to thank Dr. Gina Poe for her support through my years at the University of Michigan. Gina welcomed me into the lab and carved me a spot in her lab family. I have never met someone with the level of enthusiasm and unbridalled optimism that Gina has. Gina, thank you for sharing your passion for science and for all that you did to see me through my graduate career.

I would like to thank Dr. Victoria Booth for her constant support and encouragement. Victoria took on the role of second mentor to me, pouring over my data and editing my documents. Victoria, thank you for all the help.

I would also like to thank, Dr. Geoff Murphy, Dr. Mark Opp and Dr. Steve Maren, who complete my dissertation committee. I consider myself to be very fortunate with my committee members. Aside from our formal meetings, Mark, Geoff and Steve kept their doors open, helped with troubleshooting whenever possible, and imparted much valued future career advice. Along with many others, I appreciate

the additional time and commitment that they have shown to the Neuroscience program in general. Thank you Geoff, Mark and Steve for all the support and advice.

I would also like to thank the Sleep and Memory lab members and collaborators both past and present for the help with projects and support. In particular I would like to thank: Brett Riley, Jamie Sweigart, Lori Sagenack, David Bauer, Dr. Brooks Gross, Dr. Dinesh Pal, Howard Gritton, Dr. Theresa Bjorness, Dr. George Mashour and Bill Lipinski. Over the years Dinesh, Brett, Theresa, Bill, George, Brooks and Howard have taught me skills essential to my dissertation. And possibly more importantly, have offered those kind words that keep you going right when you're ready to quit.

I would also like to thank the anesthesiology researchers working on the 7th floor of Med Sci 1. While there are too many to name that have been a wonderful help and support, I would like to in particular thank Dr. Helen Baghdoyan, Dr. Ralph Lydic and Mary Norat. Thank you to the Anesthesiology administrative staff who have all been a wonderful help, sorting out issues with my stipend, health benefits, travelling and space issues.

I would be remiss for not expressing my gratitude to the staff in DBS for their statistical advice and help, in particular Dr. Garry Prentice, Dr. Sinead Eccles,

Pauline Cummins and Peggy Walsh. I'd also like to thank Dr. Marina Lynch for her support and advice.

Experiment 1

I would like to thank my earlier mentor and collaborators for their considerable contributions during my graduate school career: Dr. Rachael Seidler, Dr. Patti Reuter-Lorenz, Dr. John Jonides, Dr. Cindy Lustig, Dr. Robert Welsh. I learned a great deal while working with them and appreciate their help, support and advice. I am a stronger researcher and (hopefully you'll agree) scientific writer through their mentorship.

General Methods

Over the years I have asked a lot of questions and advice, I would like to thank those professors who always kept their doors open for me and again, kept me marching through my Ph.D., in particular: Dr. Rich Hume, Dr. Terri Lee, Dr. Bev Ulrich and Dr. Susan Brown.

I would also like to thank the Neuroscience administrative staff, in particular Charma Shoemaker and Valerie Smith who have always been a wonderful help and a great source of personal support, endeavoring to propagate a friendly atmosphere throughout the program.

In both labs I have worked in I have appreciated the openness to walk across or down the hall to other labs to ask questions and look for scientific advice. Thank you to those who have helped me with pesky computer issues over the years.

I have thoroughly enjoyed working with several undergraduate students over the years. In particular I would like to thank Katharine Patterson, Colleen Russell, Meghan Carroll (sleep scored my data for Chapter 3), Jung-Ho Kim (assisted in data collection, Chapter 4), Ashley Turner, Michelle Kuznia (assisted in data collection, Chapter 4), Josh Emrick and Amanda Walker. In working with each of them, I have realized the extent to which I enjoy mentoring.

I would also like to thank Dr. Ada Delaney, Aoife Foley, Dr. Ashley Bangert, Brett Fling, Dr. Dann Goble, Dr. Greg Sawicki, Dr. Javier Perez, Dr. John Perkowski, Keara Lane, Dr. Keith Gordon, Dr. Keith Sudheimer, Lillee, Dr. Mary Heng, Nick Simon, Niles C., Phoebe, Polly, Rachel Wells, Dr. Samir Khan, Dr. Shawn O'Connor, Dr. Simon Schenk, Steve Cain and Suzanne Poulton. While many have left the surrounding area – I have appreciated the frequent e-mails, notes, care packages and calls. You have all been so wonderful – I love you all.

Thank you Dr. Annie Simon for always sharing and for being there. Thank you Dr. Meghann Lloyd for letting me talk without being guarded, gathering for the early morning rugby games and for your understanding. Thank you Dr. Antoinette Domingo for docstock, for always thinking of others and for your support.

I’d like to thank my family for keeping me on track - I love you all. I’d like to thank Granny for being in the flowers; Ciara for the g-chats, the postcards and the pictures, I’ve loved being able to go on holidays with you; Anne-marie for your concern and interest and for opening your heart to us; Clare for the texts and for keeping home close by; Aunt Kathy for your constant support and encouragement; Victoria for the support, all the times we’ve shared and for always making the effort to hang out; Liz for always looking out for me, the chats and yummy desserts with cream and Peter for all the hugs. Thank you Uncle Matt for helping to make Michigan a home and for all the prayers and support – you moved me out here, and now you’re here with me as I finish. Grandma, thank you for all your calls and hugs, for sending me back with treats, your prayers and for all the years of fixing the boo-boos. Thank you for seeing me through this. Henry thank you for the love and encouragement and for being with me through all of this - I did it! Brian, thank you for always challenging me to be better, for the support and your friendship. I’ve loved being your sister. Dad, thank you for all of your advice over the years, for your edits, your visits, the rambling walks, the trips and your support. Thank you for everything and I really miss your hugs.

To the two that really walked me through and sometimes carried me through my Ph.D. – Mom and Joaquin thank you.

Joaquin thank you for my blackbird, for all the laughs and adventures, and for learning to let me cry. You have been a wonderful sounding board over the years. Thank you for helping me to see the bigger picture, for your encouragement and for seeing beyond what's in front of you.

Mom – from the late night phone calls, to all the things you've done to keep me going that are far too many to list – thank you! Thank you for always telling us we could achieve anything we wanted to and for believing in us. I couldn't imagine doing this journey without you – this Ph.D. belongs to the both of us.

Table of Contents

Dedication ii

Acknowledgements iii

List of Figures xiii

List of Tables xvi

List of Abbreviations xvii

Abstract xix

Chapter 1

General Introduction 1

- **Hippocampus** 1
- **REM Sleep** 3
- **REM Sleep and Learning** 5
- **REM Sleep Deprivation and Learning** 8
- **Reversal Learning** 16
- **REM Sleep Deprivation, Conditioning and Extinction Learning** 17
- **Prior REM Sleep Deprivation and Subsequent Learning** 19
- **REM Sleep Deprivation Techniques** 21
- **Specific Aims** 24
- **References** 26

Chapter 2

REM sleep deprivation during learning disrupts subsequent reversal learning 32

- **Abstract** 32
- **Introduction** 33
- **Methods** 38
  - Animals 38
  - REM deprivation protocol 39
  - Visual Water Maze protocol 39
  - Experiment 1 41
  - Morris Water Maze protocol 41
  - Experiment 2 45
  - Statistics 46
  - Summary 47
- **Results** 48
  - Experiment 1 48
  - Experiment 1 - Training trials during the Reversal Phase 48
  - Experiment 1 - Day 4 probe compared to Day 6 probe for platform locations 50
  - Experiment 1 - First 10 s of probe trials 50
  - Experiment 1 - Summary 51

Experiment 1 - Percent body weight 51
Experiment 2 - The effects of RD during initial spatial learning 52
Experiment 2 - The effects of rRSL on subsequent reversal learning..... 53
Experiment 2 - First 10 s of the Day 6 probe trial..... 54
Experiment 2 - Summary 55
Experiment 2 - Percent body weight 55
Discussion 55
RS restriction during the Reversal Phase and initial Learning Phase..... 57
The effects of RS restriction on subsequent reversal learning..... 62
General Discussion 67
References 89

Chapter 3

REM sleep deprivation using the inverted flowerpots method: high vs. low water level effects on sleep, recovery and learning 94
Abstract..... 94
Introduction 96
Methods 103
Animals 103
REM sleep deprivation protocol 104
Experiment 1 105
Visual Water Maze protocol 105
Morris Water Maze protocol 105
Data Analyses and Statistics..... 106
Experiment 2 107
Surgery..... 107
Sleep Recording Protocol 108
Statistics..... 110
Results 110
Experiment 1 – the Effect of High level water REM sleep deprivation on Learning 110
Experiment 1 - The Effects of High Level Water RD on Initial Spatial Learning ... 111
Experiment 1 - The Effects of High Level Water RD on the Day 4 Learning Phase Probe Trial..... 113
Experiment 1 - Summary of the Effects of RD with High Level Water During Initial Spatial Learning 114
Experiment 1 - The Effects of High Level Water RD on Subsequent Reversal Learning 114
Experiment 1 - The Effects of High Level Water RD on the Day 6 Probe Trial..... 116
Experiment 1 - Summary of the Effects of High Level Water RD on Subsequent Reversal Learning..... 116
Experiment 1 – Percent Body Weight 117
Experiment 1 - Summary of the effects of high level water RD on learning..... 117
Experiment 1 - Comparison between RD with high level water and low level water on performance effects during initial spatial training 118
Experiment 1 - Comparison between RD with high level water and low level water on performance effects during the Day 4 probe trial 118
Experiment 1 - Comparison between RD with high level water and low level water on performance effects during subsequent reversal training 119
Experiment 1 - Comparison between RD with high level water and low level water on performance effects during the Day 6 probe trial 119

Experiment 1 – Summary of the comparisons between RD with high level water and low level water on learning 120
Experiment 1 - Comparison between RD with high level water and low level water on the change in percent body weight 120
Experiment 1 – Summary of the effects of water level during RD on performance 121
Experiment 2 – Comparison of the effects of RD with either low water level and high water level RD on sleep / waking characteristics 121
Experiment 2 - Sleep / waking characteristics during and following a 6 hr deprivation period 122
Experiment 2 - Comparison of the sleep / waking characteristics during the RD and baseline periods 122
Experiment 2 - Comparison of the sleep / waking characteristics during the post-deprivation and baseline periods 123
Experiment 2 - Summary of sleep and sleep rebound results for HW Vs LW RD techniques. 125
Discussion 126
REM sleep restriction effects on initial spatial learning and subsequent reversal learning 126
Sleep / waking recordings 133
Summary 137
References 158

Chapter 4

REM sleep and Learning Following 4 Training Trials Per Day in the Morris Water Maze 161
Abstract 161
Introduction 162
Methods 168
Animals 168
REM sleep deprivation protocol 168
Visual Water Maze protocol 169
Morris Water Maze protocol 169
Statistics and data analyses 170
Results 173
The effects of rRS during the Learning Phase 174
Summary of the effects of rRS during the Learning Phase 176
The effects of rRS during the Reversal Phase 176
Summary of the effects of concurrent rRS on Reversal Learning 177
The effects of prior rRS concurrent with initial spatial learning on subsequent reversal learning 177
Summary of the effects of rRS on subsequent reversal learning 179
Body weight 180
Summary of the rRS effect on percent body weight 180
Comparison of results for control groups from the 4 training trial per day study and 12 training trial per day study 180
Control group comparisons across probe trials 181
Summary of performance for control groups in the 4 training trial per day and 12 training trial per day studies 182
Comparisons across the training trials for the rRS during initial Learning Phase groups in the 12 training trial per day and 4 training trial per day studies 182

Comparisons across the probe trials for the rRS during initial Learning Phase groups in the 12 training trial per day and 4 training trial per day studies 183
Summary of performance for rRSL groups in the 4 training trial per day and 12 training trial per day studies 184
Analyses of the relationship between Learning Phase and Reversal Phase performance 184
Summary the relationship between Learning Phase and Reversal Phase performance 186
Discussion 186
The effects of rRS during initial spatial learning 187
The effects of rRS during reversal learning 192
The effects of rRS during initial spatial learning on subsequent reversal learning 193
General Discussion 197
References 221

Chapter 5

General Discussion 228
Summary 228
RS effects on initial Spatial Learning 232
RS effects on Reversal Learning 239
Subsequent Reversal Learning 240
RS Restriction Windows 242
Effects of RS disruption that can affect behavioral outcome 243
The Appropriateness of Currently Used Tasks 244
Is REM sleep essential for learning? 248
Future Directions 251
Conclusion 252
References 254

Appendix

The Effects of REM Sleep Restriction on the T-maze 257
Introduction 257
Learning 257
Sleep 258
Methods 259
Animals 259
REM sleep deprivation protocol 260
Visual Water Maze protocol 260
Habituation protocol 260
T-maze 261
Statistics 263
Results 263
Summary 265
Discussion 265
Discussion concerning the differing results between my current study and prior work in the lab for the number of correct trials 266
Discussion of the group difference in the number of trials a rat failed to leave the start arm 267
Summary 270
References 280

List of Figures

Figure 2.1 The inverted flower pot technique for REM sleep deprivation. ..69
Figure 2.2 Morris water maze. ..70
Figure 2.3 Experiment 1 protocol..71
Figure 2.4 Experiment 2 protocol..72
Figure 2.5 Experiment 1: Latency. ..73
Figure 2.6 Experiment 1: Pathlength. ..74
Figure 2.7 Experiment 1: Cumulative distance from the platform. ..75
Figure 2.8 Experiment 1: First 5 s of trials for Cumulative distance from the platform across the Reversal Phase. ..76
Figure 2.9 Experiment 1: Percent time spent in target quadrant during the probe trials. ..77
Figure 2.10 Experiment 1: Number of platform crossings during the probe trials. ..78
Figure 2.11 Experiment 1: Percent body weight ..79
Figure 2.12 Experiment 2: Latency ..81
Figure 2.13 Experiment 2: Cumulative distance from target platform ..82
Figure 2.14 Experiment 2: First 5 s of Cumulative distance from target platform ..83
Figure 2.15 Experiment 2 Percent time spent in target quadrant during the probe trials ..84
Figure 2.16 Average proximity to target platform during the probe trial ..85
Figure 2.17 Number of platform crossings during the probe trial ..86
Figure 2.18 Summary of the results fro Experiments 1 and 2. ..88
Figure 3.1 Depiction of the REM sleep deprivation chambers ..140
Figure 3.2: Experiment 1 protocol ..141
Figure 3.3 - Experiment 2 protocol ..142
Figure 3.4 Latency to platform ..143
Figure 3.5 – Cumulative distance from the platform ..144
Figure 3.6 First 5 s of cumulative distance from the platform ..145
Figure 3.7 Velocity ..146
Figure 3.8 Percent time spent in quadrant within the first 10 s of the probe trials ..147
Figure 3.9 Number of Platform Crossings within the first 10 s of the probe tests ..148
Figure 3.10 Percent time spent in the target quadrant during the 60 s Probe trial ..149
Figure 3.11 Average proximity to platform during the 60 s Probe trial ..150
Figure 3.12 Percent Body Weight ..151

Figure 3.13 Quiet sleep during the 6 hr deprivation period..... 152
Figure 3.14 REM sleep and transitions to REM sleep during the 6 hr deprivation..... 153
Figure 3.15 Quiet sleep during the 2-4hr post-deprivation period..... 154
Figure 3.16 REM sleep and transitions to REM sleep during the 2-4hr post-deprivation period..... 155
Figure 4.1 Experiment protocol..... 201
Figure 4.2 Percent time in target quadrant during the first 10 s of the Probe trial..... 202
Figure 4.3 Average proximity to the target platform during the first 10 s of the Probe trial..... 203
Figure 4.4 Percent time in target quadrant during the 60 s of the Probe trial..... 204
Figure 4.5 Average proximity to the target platform during the 60 s of the Probe trial..... 205
Figure 4.6 The first 5 seconds of cumulative distance from the target platform during the Learning Phase..... 206
Figure 4.7 Latency to platform..... 207
Figure 4.8 Percent time in target quadrant during the 60 s of the Probe trial..... 208
Figure 4.9 Average proximity to the target platform during the 60 s of the Probe trial..... 209
Figure 4.10 Percent body weight based on Day 1 body weights..... 210
Figure 4.11 Percent body weight based on Day 4 body weights..... 211
Figure 4.12 Comparison across studies for the first 5 s of cumulative distance..... 212
Figure 4.13 Comparison across studies for the first 5 s of cumulative distance..... 213
Figure 4.14 Comparison across studies for time spent in target quadrant during the probe trials..... 214
Figure 4.15 Comparison across studies for the number of platform crossings during the probe trials..... 215
Figure 4.16 Comparison across studies for average proximity to platform during the probe trials..... 216
Figure 4.17 Summary of results of 4 training trials per day study. 219
Figure 4.18 Summary of Discussion..... 220
Figure A.1 Total number of correct trials performed on the T-maze..... 272
Figure A.2 Total number of incorrect trials performed on the T-maze..... 273
Figure A.3 Total number of trials performed on the T-maze..... 274
Figure A.4 The number of correct trials as a percent of the total number of trials performed on the T-maze..... 275
Figure A.5 The number of incorrect trials as a percent of the total number of trials performed on the T-maze..... 276
Figure A.6 Trial length..... 277
Figure A.7 The number of correct trials performed by each individual rat on the T-maze..... 278

Figure A.8 The total number of trials performed by each individual rat on the T-maze..... 279

List of Tables

Table 2.1 Summary of the behavioral results for rRS concurrent with the Reversal Phase as compared to controls……..…….80
Table 2.2 A Summary of the effects of rRS during the Learning Phase…87
Table 2.2 B Summary of the effects of prior rRS on Reversal Phase performance……...87
Table 3.1 Summary of the deprivation chambers used in learning and REM sleep studies……139
Table 3.2 A Summary of performance differences resulting from RD with high level water as compared to controls during the Learning Phase……..156
Table 3.2 B Summary of performance differences resulting from prior RD with high level water as compared to controls on the subsequent Reversal Phase performance..…..…….156
Table 3.3 A Summary of performance differences resulting from RD with high level water as compared to low level water during the Learning Phase…….157
Table 3.3 B Summary of performance differences resulting from prior RD with highlevel water as compared to low water level on the subsequent Reversal Phase performance……..157
Table 4.1 A Summary of the behavioral results for rRSL compared to controls for the Learning Phase……..…….217
Table 4.1 B Summary of the behavioral results for rRSL compared to controls for the subsequent Reversal Phase…….……...….217
Table 4.2 Summary of the behavioral results for rRSR compared to controls for the Reversal Phase……..218

List of Abbreviations

BDNF	Brain Derived Neurotrophic Factor
CON	Control group
CONL	Control group for Learning Phase manipulations
CONM	Mixed Control group (young and old)
CONR	Control group for Reversal Phase manipulations
EEG	Electroencephalogram
EMG	Electromyogram
HW	REM sleep deprived with high level water group
Learn	Learning Phase
LTP	Long-term Potentiation
LW	REM sleep deprived with low level water group
mPFC	Medial Prefrontal Cortex
NE	Norepinephrine
OCON	Old Control group
OR	Old Rats
OrRS	Old REM Sleep Restricted group
RD	REM sleep Deprivation
REM	Rapid Eye Movement
Rev	Reversal Phase

rRS	REM Sleep Restricted
rRSL	REM Sleep Restricted during the Learning Phase group
rRSM	REM Sleep Restricted Mixed group (young and old)
rRSR	REM Sleep Restricted during the Reversal Phase group
rRSRev0-6	rRSR in the 0 - 6 hrs time window following training group
rRSRev6-12	rRSR in the 6 – 12 hrs time window following training group
RS	REM sleep
SEM	Standard Error of the Mean
TpD	Training trials Per Day
YCON	Young Control group
YR	Young Rats
YrRS	Young REM Sleep Restricted group

Abstract

Typically, when REM sleep restriction is applied during learning (concurrent learning) performance is impaired. It is unclear how REM sleep restriction can alter other forms of spatial learning (e.g. reversal learning). For my dissertation I studied the effect of concurrent REM sleep restriction on both initial spatial learning and reversal learning, as well as the effect of prior REM sleep restriction on subsequent reversal learning in the Morris water maze. When using 12 training trials per day, I found that REM sleep restriction concurrent with either initial spatial learning or reversal learning were not affected. However, prior REM sleep restriction resulted in performance deficits during subsequent reversal learning. When using 4 training trials per day, I found that again REM sleep restriction did not affect concurrent reversal learning. In contrast, REM sleep restriction resulted in the typically reported deficits during initial spatial learning. Additionally prior REM sleep restriction was associated with performance enhancements during subsequent reversal learning. My results suggest that concurrent reversal learning is protected from the detrimental effects of REM sleep restriction. Across my dissertation, I identify an interactive relationship between the number of training trials, or learning load, and REM sleep restriction to modulate performance. Though REM sleep restriction does appear to alter

learning, the performance deficit may not be measurable during the initial learning experience if the training session is sufficient to produce near asymptotic performance, but only on subsequent learning events. These behavioral findings support the hypothesis that REM sleep facilitates both the consolidation of incomplete learning and the desaturation of neuronal networks for subsequent learning purposes. Lastly, my studies emphasize the inability to ascertain the role of REM sleep when generalizing across learning, even when limiting the focus to spatial learning.

Chapter 1

General Introduction

As far back as the mid 17th century, Descartes theorized over the link between sleep and learning, postulating that though the brain is conscious throughout sleep it is unable to create new memories. In fact, philosophers and artists (e.g. Shakespeare, late 16th century) have been focused on sleep far longer than recorded accounts in the biological sciences (the first pubmed reference for sleep is from 1881 (Regnard, 1881)). Today, in an ever-increasing sleep-deprived society, knowing the impact of the lack of sleep is imperative. Researchers have been able to describe the impact of sleep deprivation on various emotional states, attention and response times. While several postulate over the role of sleep for learning, nearly four centuries later we still cannot verify Descartes' original theories. The goal of my dissertation is to further our understanding of the relationship between learning and rapid eye movement sleep.

Hippocampus

The hippocampus is located in the temporal lobe and named for its seahorse-like

shape. It is widely accepted that the hippocampus is involved in various types of learning (e.g. Meissner, 1966; Kesner, Evans, & Hunt, 1987), including the types of learning explored in this dissertation work. Hippocampal activity has been associated with spatial learning (e.g. Barnes, 1988), the formation of episodic memories (e.g. Nadel & Moscovitch, 1997) and the inhibition of previously learned responses (e.g. Kimble, 1968). Patient H.M. was unable to form new episodic memories following surgery-related bilateral damage to his hippocampus, though older episodic memories remained intact which led to studies indicating that memories initially depending on the hippocampus have a graded consolidation profile to the neocortex (e.g. Kim & Fanselow, 1992). O'Keefe and Dostrovsky (1971) indicated that hippocampal activity was altered depending on the location of the rat in space. In rats with hippocampal lesions, both spatial learning and reversal spatial learning are impaired (Samuels, 1971). With the support of numerous studies since those mentioned above (e.g. Morris, Hagan, & Rawlins, 1986; O'Keefe, 1993; Whishaw & Tomie, 1997) it has been determined beyod a reasonable doubt that the hippocampus has a role in spatial learning. The identification of hippocampal place cells which activate in relation to specific locations in space further supported the relationship between hippocampal cell activity and spatial location learning (O'Keefe, 1976). This dissertation work focuses on spatial learning and its reversal, both of which depend on the hippocampus, and on REM sleep, which has been shown to affect hippocampus-dependent learning (for review: Smith, 1995).

Long-term potentiation is a neural mechanism thought to be the synaptic basis for learning. Long-term potentiation (LTP) is the strengthening of the synaptic connection between neurons and occurs readily in the hippocampus, where it was first observed (Bliss & Lomo, 1973). The LTP process initiates the transcription of a number of immediate early genes such as *zif* 268 (Cole et al., 1989), which act to further the LTP response e.g. through creation of synaptic protiens. LTP processes are impaired by REM sleep deprivation (Davis, Harding, & Wright, 2003; Ishikawa, et al., 2006; E. Y. Kim, Mahmoud, & Grover, 2005; McDermott, Hardy, Bazan, & Magee, 2006; McDermott, et al., 2003; Ravassard, et al., 2009) and it is possible that the learning effects I describe in this dissertation are mediated through REM sleep deprivation effects on LTP as well as on its reversal, called depotentiation, described later.

REM Sleep

Typically sleep can be divided into two major components: rapid eye movement (REM) sleep and non REM sleep. Non REM sleep itself is comprised of two main components: quiet sleep (QS) and transitions to REM sleep (TR). Each of the sleep / waking states can be differentially described by specific characteristics within recordings of frontal and parietal electroencephalography and neck muscle electromyography. REM sleep (RS) is characterized by 1) muscle atonia; 2) movements of the eyes, inner ear muscles or, in insects, movement of the antennae; and 3) high frequency, low amplitude activity in the frontal electroencephalogram (EEG) and steady, high amplitude activity in the theta

frequency band (~ 4 – 10 Hz) in EEG from parietal sources. This theta wave is also present in the parietal EEG during active waking, but is then coincident with voluntary movement and thus higher electromyogram (EMG) activity, which easily distinguishes itself from REM sleep.

REM sleep is associated with sharp declines in activity of both the Locus Coeruleus and the Dorsal Raphe. Both the Locus Coeruleus and the Dorsal Raphe have minimum activity during REM sleep as opposed to non-REM sleep and waking states (Aston-Jones & Bloom, 1981; McGinty & Harper, 1976). The Locus Coeruleus is the principal source for the release of norepinephrine throughout the forebrain, and the Dorsal Raphe is a primary source for the release of serotonin. The release of both norepinephrine (Shouse, Staba, Saquib, & Farber, 2000) and serotonin (Iwakiri, Matsuyama, & Mori, 1993; Park, et al., 1999; Penalva, et al., 2003; Portas & McCarley, 1994) are at minimum levels during REM sleep as compared to non-REM sleep. In contrast, acetylcholine is increased (Jasper & Tessier, 1971; Vazquez & Baghdoyan, 2001) during REM sleep as compared to non-REM sleep. Therefore, while muscle atonia and a hippocampal theta rhythm are characteristic of REM sleep, so is a unique change in the neurochemical milieu in the brain.

There is much debate over the function of REM sleep (for review see Winson 1993). Among the possible roles of REM sleep are: thermoregulation, to provide a window of increased vigilance while asleep, to allow replenishment of

neurotransmitters or their recepetors, or to facilitate learning. For the purposes of this dissertation I will be focusing on the possible role of REM sleep for learning. In particular, for my dissertation I will focus on the interaction of REM sleep modulation and hippocampal-dependent learning, e.g. spatial learning and its reversal. In the next section I will describe the currently understood relationship between spatial learning and REM sleep modulation.

REM Sleep and Learning

Generally, the role of RS is a much-debated topic, and even more so with respect to the role of RS for learning (for example: Hobson & Pace-Schott, 2002; Rauchs, Desgranges, Foret, & Eustache, 2005; C. Smith, 1995; Stickgold & Walker, 2005; Vertes, 2004; Vertes & Siegel, 2005). Until recently, a key argument against the role of RS for learning was in the expression of RS across phylogeny with respect to the described intelligence of those species. For example, dolphins have little RS and are among the cognitively advanced creatures, while the platypus has a great deal of RS and is one of the least intelligent species (for review: Siegel, 2001). However, in a recent review (Lesku et al., 2009) it was suggested that when percent time in RS is compared with the amount of encephalization across species, a positive correlation was identified. As greater encephalization is thought to be associated with greater cognitive ability (for review: Lesku et al., 2009) these results suggest that the more cognitively able species spend an increased percent time in RS. This argument supports a potential link between RS and intelligence. Further, the amount of RS

in the learning dolphin (or many other animals) has never been measured and it may be the increase in RS that has a role in learning as opposed to basal levels of RS.

Arguments supporting the role of RS for learning include: increases in RS following learning, expression of a gene associated with learning during RS, neural reactivation of the learned experience during RS and performance deficits resulting from RS deprivation (RD) or RS restriction (rRS). These arguments are now discussed in turn.

Following a learning experience, increases in the amount of subsequent RS have been observed across a range of species. Examples of these include increases in REM or paradoxical sleep following imprinting in newborn chicks (Solodkin et al., 1985), spatial learning in rats (C. Smith & Rose, 1997), avoidance learning in rats (Bramham, Maho, & Laroche, 1994; Fishbein, Kastaniotis, & Chattman, 1974; Mavanji & Datta, 2003; Portell-Cortes, Marti-Nicolovius, Segura-Torres, & Morgado-Bernal, 1989; C. Smith & Butler, 1982; C. Smith & Lapp, 1986; C. Smith & Wong, 1991; C. Smith, Young, & Young, 1980), positive reinforcement conditioning in cats (Lecase, 1976) and learning Morse code in humans (Mandai et al., 1989). The observed improvements in performance following the increase in RS are positively correlated with the amount of RS increase (C. Smith & Wong, 1991).

In a study to produce long-term potentiation (LTP, a mechanism of learning) within the hippocampus, LTP could be induced during waking and RS but not during non-RS (Bramham & Srebro, 1989). Further evidence for the role of RS and learning came from a study of zif-268, an extrahippocampal gene associated with hippocampal LTP (Ribeiro, et al., 2002), where zif-268 was increased throughout specific areas of the brain (e.g. amygdala, auditory, entorhinal, motor, and somatosensory cortices) following LTP induction, both during waking and the first few RS episodes. However, when hippocampal activity was blocked during these RS episodes, the increases in extrahippocampal zif-268 were not observed. Without hippocampal activity during RS, the increases in extrahippocampal zif-268 were not observed suggesting a RS specific communication between the hippocampus and extrahippocampal brain regions such as the amygdala and various cortical areas.

The link between RS and hippocampal activation for learning was further supported when hippocampal place cell firing was recorded following training on both a novel and a familiar maze. As described earlier, a hippocampal place cell is a cell within the hippocampus whose activation is associated with specific positions in space (e.g. left corner of a maze). A place cell can be active or fire at specific phases (the peaks or troughs) of the theta wave within the hippocampus during waking and REM sleep. Previous literature (Poe, Nitz, McNaughton, & Barnes, 2000) has shown that hippocampal place cells fire at the peaks of the hippocampal theta EEG (theta peaks) in both novel and familiar mazes.

However, during REM sleep the pattern of place cell activation changes in relation to the theta phase. This is of relevance as hippocampal cell activity at theta peaks versus theta troughs has different implications. Hippocampal cellular firing at theta peaks is associated with inducing LTP (Hölscher et al., 1997). In contrast, hippocampal cellular firing at theta troughs is associated with cellular depotentiation (Hölscher et al., 1997). Depotentiation is the resetting of previously potentiated synapses, similar to the opposite role of LTP. Therefore, it is thought that depontentiation is involved with 'unlearning' or removal of a learned experience from within the hippocampus. It was proposed that REM sleep may have a role in reverse learning or the unlearning of previously learned responses (Crick & Mitchison, 1983; Gaarder, 1966; Newman & Evans, 1965). The change in the theta phase of hippocampal cell activity during REM sleep further suggests that REM sleep has a role in the learning process. REM sleep has also been associated with maintaining the temporal aspect of performing on the maze during task replay across the night (Louie & Wilson, 2001) as observed with hippocampal place cell activation throughout quiet sleep and RS.

REM Sleep Deprivation and Learning

One way to determine the importance of RS for learning is to disrupt RS. Two ways of doing this is by depriving RS for short amounts of time leading to RS restriction (rRS) or complete RS deprivation (RD). While RD lasting 24 -72 hrs has been typically used when studying the role of RS, rRS is more typical of human daily living. Typically, similar effects on learning have been shown for

both RD and rRS (e.g. Pearlman, 1973; C. Smith & Rose, 1996; Youngblood, Zhou, Smagin, Ryan, & Harris, 1997). See the section “REM Sleep Deprivation Techniques” in this chapter for an description of the typical RD techniques used.

Both prolonged RD durations (24 - 120 hrs: Davis, Harding, & Wright, 2003; Kim, Mahmoud, & Grover, 2005; McDermott, et al., 2003; Ravassard, et al., 2009) and a shorter bout of RD (4 hrs: Romcy-Pereira & Pavlides, 2004) impaired induction of hippocampal LTP. The short bout of RD resulted in an impairment of LTP 48 hrs later (Romcy-Pereira & Pavlides, 2004), compared to an observed impairment at 24 hrs post-RD as was seen with the longer RD periods (Davis, et al., 2003; McDermott, et al., 2003; Ravassard, et al., 2009).

The impact of RD during behavioral studies has been variable, though it often results in performance deficits (for review: McGrath & Cohen, 1978; C. Smith, 1985, 1995; Vertes & Eastman, 2000). Several researchers have found that in the animal model, in particular using rats, that REM sleep restriction or REM sleep deprivation impaired spatial learning (Bjorness, Riley, Tysor, & Poe, 2005; Li, et al., 2009; C. Smith & Rose, 1996, 1997; C. T. Smith, Conway, & Rose, 1998; Wang, et al., 2009; Youngblood, et al., 1997), conditioning (Fu, et al., 2007; Pearlman, 1973; Silvestri, 2005) or avoidance learning (for review: McGrath & Cohen, 1978). While many reported that RD or rRS resulted in performance deficits, several did not (for review McGrath & Cohen, 1978; Smith, 1995; Vertes & Eastman 2000). It was postulated that performance was

protected from the REM sleep manipulation as a result of methodological differences (for review McGrath & Cohen, 1978; Smith, 1995; Vertes & Eastman 2000). These methodological differences include inappropriate deprivation techniques, inappropriate controls, near asymptotic learning being reached prior to the manipulation or inappropriate timing of the RS manipulation. In contrast, others have postulated that the observed deficits in performance are not indicative of the effects of RD on learning, but are a result of RD technique-associated impairments on performance itself (Vertes and Eastman, 2000). However, shorter periods of RD would not lead to strong side-effects which could impact learning. Thus any observed performance deficits with short periods of RD are likely an effect on learning (Vertes and Eastman, 2000).

Several studies have indicated that behavior is only sensitive to a RS manipulation when applied at certain time points (Smith & Rose, 1996, 1997; Fu, et al., 2007). Therefore, when a short period of RD was applied throughout the RS sensitive window a performance deficit was observed. When an identically long period of RD was applied outside of the RS sensitive window, no performance deficit was observed (Smith & Rose, 1996, 1997; Fu, et al., 2007). The sensitive RS sensitive window tends to be immediately following the training period, though the timing of the RS sensitive window may be affected by the amount of training (Smith and Rose, 1996, 1997).

Few studies have been performed on humans to test the effects of REM sleep deprivation on learning. Previous reports have indicated that learning is sensitive to sleep loss or sleep disruption (e.g. Stickgold & Walker, 2005; Yoo, Hu, Gujar, Jolesz, & Walker, 2007). In the human literature, some researchers have indicated that REM sleep facilitates procedural learning or processing of newly formed emotional memories (Fogel, Smith, & Cote, 2007; Smith, 2001; Walker & van der Helm, 2009), while others have indicated that REM sleep does not facilitate procedural learning (Genzel, Dresler, Wehrle, Grozinger, & Steiger, 2009; Hornung, Regen, Danker-Hopfe, Schredl, & Heuser, 2007). Therefore, in humans, hippocampal-dependent learning may be independent of RS manipulations.

In this dissertation, I will be focusing specifically on spatial learning in the rat model, which is thought to be dependent on the hippocampus. As you will see, this is a more stable model for studying the effects of RS modulation and learning. RD and spatial learning has been studied in rats using a variety of learning tasks, including the Morris water maze (e.g. Li, et al., 2009; Ruskin, Dunn, Billiot, Bazan, & LaHoste, 2006; C. Smith & Rose, 1996, 1997; Wang, et al., 2009; Youngblood, et al., 1997), the radial arm maze (C. T. Smith, Conway, & Rose, 1998) and the Poe 8-box maze (Bjorness, et al., 2005). In all these tasks, an RD associated performance deficit was identified irrespective of the duration of RD (4 - 72 hrs).

In one study of the effects of prolonged RD on both spatial reference and working memory (Youngblood, et al., 1997), animals were trained in the Morris water maze for 4 trials per day for 4 days. RD was started ~ 24 hrs before exposure to the Morris water maze and continued throughout the study. Though spatial working memory was unaffected, spatial reference memory was impaired with RD. Rats that underwent RD lost significantly more body weight across the study than controls and swam faster in the Morris water maze from Day 2 onwards. Unfortunately with the paradigm utilized in the Youngblood et al. study, it is not possible to differentiate the effects of RD on acquisition versus consolidation of learning. Manipulations prior to performing the maze should target acquisition of learning, while manipulations following performance on the maze would target consolidation of learning. As RD commenced prior to their first learning session and no deficits were identified on Day 1, these results are suggestive of an effect of RD on consolidation, though it is not possible to clearly determine.

Unlike the Youngblood et al. (1997) study, other studies using prolonged RD have attempted to target either acquisition of learning or consolidation singly. In a study using a similar paradigm to Youngblood et al. (1997) who used ~ 24 hrs of RD per day for 4 days, Ruskin et al. (2006) administered 72 hrs of RD prior to performing the Morris water maze. In contrast to the findings of Youngblood et al. (1997), Ruskin et al. found RD-associated deficits in spatial working memory as opposed to spatial reference memory. This work suggested that prolonged RD prior to acquisition disrupted spatial working memory.

In studies focused on the effects of prolonged RD on learning consolidation (Li, et al., 2009; Wang, et al., 2009), RD was administered for 72 hrs after either 3 or 5 days of training (4 trials per day) in the Morris water maze, respectively. Both studies described a performance deficit in allocentric learning. These studies (Li, et al., 2009; Wang, et al., 2009) indicate that the effects of prolonged RD are sufficiently strong to disrupt consolidation even after 2 or 4 prior days of consolidation.

Shorter bouts of RD to target consolidation of learning have also resulted in performance deficits, indicating that RD disrupts consolidation. Specifically, deficits in allocentric learning at the start of the second day of training in the Morris water maze were identified following 4 hrs of RD (C. Smith & Rose, 1996, 1997). Together, these studies indicate that irrespective of the duration of RD (4 – 72 hrs) consolidation of learning is disrupted in the Morris water maze (Li, et al., 2009; C. Smith & Rose, 1996, 1997; Wang, et al., 2009).

Other spatial learning tasks have also been used to determine the effects of RD following daily training such as the 8 – arm maze (C. T. Smith, et al., 1998) and the Poe 8 – box maze (Bjorness, et al., 2005). In the 8 – arm maze study, 4 hrs of daily RD following daily training for 10 days resulted in impaired spatial reference memory but not spatial working memory throughout the study (C. T. Smith, et al., 1998). This suggests that the impairments seen during the

Youngblood et al. study (1997) may have been the result of the RD following training as opposed to the deficits observed as a result of RD prior to training. Using the Poe 8-box maze, 4 hrs of daily RD following daily training for 15 days, resulted in impaired spatial learning (Bjorness, et al., 2005). Together, these studies (Bjorness, et al., 2005; Li, et al., 2009; C. Smith & Rose, 1996, 1997; C. T. Smith, et al., 1998; Wang, et al., 2009) indicate that RD following training on a spatial learning task impairs performance irrespective of task and RD duration in the rat model. Evidence also suggests that RD following training results in the reliance on non-hippocampal strategies to solve the task (Bjorness, et al., 2005).

Both long and short bouts of RD impair both LTP within the hippocampus and disrupt performance when RD is administered prior to or following training on a spatial learning task. Therefore, RS appears to have a role in hippocampal dependent learning processes.

The majority of the RD or rRS and spatial learning studies in the Morris water maze relied primarily on latency to platform as the measurement of performance. Some other measures considered included pathlength (Li, et al., 2009; Wang, et al., 2009; Youngblood, et al., 1997), number of target quadrant entries (C. Smith & Rose, 1996) and number of quadrant entries (Beaulieu & Godbout, 2000). Of those that actually did use the more spatial learning sensitive probe trial to test for learning, only time spent in target quadrant was reported (Wang, et al., 2009). Although some measures have been described as being more sensitive and

robust, such as Gallagher's cumulative distance from the platform during training and Gallagher's average proximity to the platform during probe trials (Gallagher, Burwell, & Burchinal, 1993; Hodges, 1996; Maei, Zaslavsky, Teixeira, & Frankland, 2009), they have not yet been used to assess differences in performance resulting from manipulations of RS. Further, thus far, only measures computed over the entire trial length in the Morris water maze have been reported within the RD literature, rather than determining if RD-associated offsets in performance were present in the initial portions of a trial, more telling of effects on reference memory, that may no longer be detectable later in the trial.

Previous work has shown that the number of training trials prior to short bouts of RD can alter the RD sensitive period (C. Smith & Rose, 1996, 1997). A RD sensitive period is a time period during which RD results in associated subsequent performance deficits. However, if RD is administered outside of this sensitive window, no associated subsequent performance deficits will be observed. This suggests that there may be an interactive effect between level of learning load (or number of trials) and the timing of learning-associated processes in RS. These studies also indicated that unlike the rRS and land-based appetitive spatial tasks (Bjorness, et al., 2005; C. T. Smith, et al., 1998) which indicated rRS-associated performance deficits throughout the study, performance in the Morris water maze may only be affected at the start of the second day of as opposed to throughout the duration of the study (C. Smith & Rose, 1996). Though the timing of the deficit in latency was observed at the

same time point irrespective of learning load in the Morris water maze, (C. Smith & Rose, 1996, 1997), only the lighter learning load was tested across days (C. Smith & Rose, 1996). Therefore, it is currently unclear whether a heavy load of learning across multiple days in the Morris water maze with concurrent rRS immediately following training would lead to lasting performance deficits similar to those seen with the relatively heavy trial loads on the land-based tasks (Bjorness, et al., 2005; C. T. Smith, et al., 1998).

Reversal Learning

Current theories on hippocampal-dependent spatial learning posit that when solving a spatial task, a mental map is created based on the target location and available environmental cues. When the target location is altered, but all environmental cues remain fixed, such a mental map would need to be altered or an alternative map generated. This phenomenon of relearning is referred to as reversal learning (for review: Whishaw, 1998). Therefore reversal learning requires an element of unlearning (for review: van der Meulen, et al., 2003).

Although the effects of RD or rRS on the reversal of spatial learning have not yet been studied, reversal spatial learning has been used in a range of other studies using the Morris water maze and the 8-arm maze. Similar to spatial learning, reversal learning is a hippocampal-dependent task (Whishaw & Tomie, 1997). Some studies suggest that reversal learning takes fewer trials to learn as compared to initial spatial learning (Guzowski, Setlow, Wagner, & McGaugh,

2001; Whishaw & Tomie, 1997) and may be less susceptible to hippocampal damage (Conrad, Galea, Kuroda, & McEwen, 1996), where damage to the dentate gyrus resulted in impaired spatial learning but not subsequent reversal learning. There was no effect of cell loss within the dentate gyrus on reversal learning alone, when the initial spatial learning task was acquired with an intact hippocampus. However, other studies suggest that reversal learning may be more vulnerable to altered hippocampal activity (Cirulli, Berry, & Alleva, 2000; Cirulli, Berry, Chiarotti, & Alleva, 2004; Pouzet, et al., 1999).

REM Sleep Deprivation, Conditioning and Extinction Learning

While reversal learning is the learning of a new response to a stimulus (e.g. moving to a new target location when exposed to a maze), extinction learning is the uncoupling of the response from the stimulus. Although reversal learning has not yet been studied for its relationship with RS in the rat model, the effects of RS have been studied on extinction learning of conditioned taste aversion, conditioned bar pressing and fear conditioning. Though reversal learning and extinction learning may rely on similar brain structures or mechanisms (e.g. van der Meulen, et al., 2003), the effects of RD or rRS on reversal learning may differ to those seen with extinction learning.

Interestingly, RD did not have a uniform effect on extinction of these various learning types. Specifically, when RD (~ 20 hrs per day for 3 days) was administered immediately following extinction training on a conditioned bar press

response task in rats, RD improved the rate of extinction (Pearlman, 1973). Therefore, these results suggest that RD enhances extinction learning for conditioning bar pressing.

In contrast, in an experiment to determine the effects of concurrent RD on the extinction of both cued fear conditioning and contextual fear conditioning, 6 hrs of RD was applied directly following the first session of extinction training (Fu, et al., 2007). While concurrent RD had no effect on the extinction of contextual fear conditioning, the extinction of cued fear conditioning was delayed. As with spatial learning tasks, a RD sensitive window was identified immediately following training rather than delayed by 6 hrs for extinction training (Fu, et al., 2007). A similar deficit to the extinction of fear conditioning was observed when 24 hrs of RD was administered prior to extinction training of a conditioned taste aversion task (Venkatakrishna-Bhatt, Bures, & Buresova, 1979). This task was carefully controlled to avoid a potential effect of RD on the consolidation of the taste aversion conditioning itself. Together these studies (Fu, et al., 2007; Venkatakrishna-Bhatt, et al., 1979) indicate that RD could impair extinction of both cued fear conditioning and conditioned taste aversion. Thus concurrent RD with extinction training may or may not lead to deficits in extinction, but is there a uniform response with subsequent learning, e.g. reversal learning?

Prior REM Sleep Deprivation and Subsequent Learning

The effect of RD on subsequent extinction learning was tested when RD was administered during a pre-extinction experience in the extinction environment prior to extinction training (Pearlman, 1973). The basis of the experiment was that pre-exposure to the conditioned environment in the absence of the reward would increase the rate of subsequent extinction. When RD was administered immediately following the pre-exposure, latent extinction learning was impaired as compared to normal sleeping controls following the pre-extinction experience. In fact, the REM deprived rats acted similarly to those that had not undergone the pre-exposure event at all. The impact of RD on latent extinction was observed with both prolonged RD (~ 20 hrs per day, for 3 days) and short bouts of RD (5 hrs) immediately following the pre-extinction experience (Pearlman, 1973). Therefore in a paradigm that prior exposure to the extinguishing environment, or pre-extinction, should have resulted in faster extinction training of the conditioned bar pressing task, both long and short bouts of RD resulted in impaired subsequent extinction training, as if the pre-extinction learning period had never occurred.

In a fear conditioning study, the effect of RD concurrent with fear conditioning was tested for its effects on subsequent extinction. When RD (6 hrs) was administered immediately following fear conditioning in rats, there was no impairment in fear conditioning (Silvestri, 2005; Silvestri & Root, 2008). However, subsequent extinction of the cued fear conditioning was delayed (Silvestri, 2005;

Silvestri & Root, 2008), though not for the extinction of contextual fear conditioning (Silvestri, 2005).

These studies indicate that RD can disrupt subsequent extinction of both conditioned bar pressing and cued fear conditioning. These deficits in latent extinction were found following both short and long periods of RD, similar to the findings in the spatial learning and RD literature. It is currently unknown how RD will affect subsequent reversal of a spatial learning task in the rat model.

As previously described, the level of norepinephrine is low within the hippocampus during RS, compared to levels during waking and nonREM sleep. In a spatial learning study using the 8-arm maze (Harrell, Barlow, Miller, Haring, & Davis, 1984) the effect of the absence of hippocampal norepinephrine on both spatial learning and reversal learning was assessed. Spatial learning was unaffected by with the administration of 6-hydroxydopamine (to remove noradrenergic neurons) while reversal learning was enhanced. This suggests that the presence of RS, when norepinephrine is absent, could enhance reversal learning, and therefore RD, causing maintenance of high norepinephrine levels, could result in performance deficits equal to or worse than those RD-associated deficits typically seen during spatial learning, if reversal learning is indeed more sensitive to noradrenergic levels. To further support this theory, it is thought that RD disrupts medial prefrontal cortex (mPFC) activity based on deficits seen with the frontal cortex dependent behavioral task used (Beaulieu & Godbout, 2000; Le

Marec, et al., 2001). Both reversal learning (de Bruin, Sanchez-Santed, Heinsbroek, Donker, & Postmes, 1994) and extinction learning (Morgan, Romanski, & LeDoux, 1993) are impaired when the mPFC is damaged, or when mPFC activity is blocked (van der Meulen, et al., 2003). Reversal of spatial learning was shown to be vulnerable to lesions of the mPFC as compared to initial spatial learning (de Bruin, et al., 1994).

REM Sleep Deprivation Techniques

There are several techniques currently used to specifically target and deprive rats of RS, while attempting to preserve the remaining sleep / waking stages. The techniques typically used for this are the inverted flowerpot method (Jouvet et al., 1964), gentle handling and disk over water (Bergmann, et al., 1989). Gentle handling works by gently waking the subjects by touch when they enter RS. However, this requires the animals or subjects to be fitted for on-line EEG and EMG measurements in order to determine when to disrupt their entrance into RS. The disk over water technique consists of a disk suspended over water. The rat is placed on the disk which rotates when the rat enters RS to wake them, again requiring on-line EEG and EMG measurements to detect the onset of RS.

The inverted flowerpot technique utilizes the onset of muscle atonia with RS to disrupt RS. This technique does not require the animals to be tethered for concurrent EEG and EMG measurements. Depending on the research lab, the subject is placed on either a single or multiple inverted flowerpots within a

chamber. The chamber is filled with water to a level typically either as low as 2 cm at the bottom or as high as within 1 cm of the flowerpot platform. At the onset of muscle atonia associated with RS, the subject wakes as they start to fall from the platform. For this technique to work, it is imperative that the platform-to-subject size meet a ratio that allows the animal to be comfortable and sleep, but small enough that they can not assume a fully supported sleep atonia posture (Hicks, Okuda, & Thomsen, 1977; McGrath & Cohen, 1978).

Unfortunately, each of these RD techniques can be associated with increased stress levels. A number of attempts have been made with the inverted flowerpot technique, in particular, to lessen the effects of stress by altering characteristics of the RD chamber itself.

Of these three possible RD techniques, the inverted flowerpot technique is the predominantly used method to administer RD during RS and learning studies (Beaulieu & Godbout, 2000; Bjorness, et al., 2005; Davis, et al., 2003; Fu, et al., 2007; Kim, et al., 2005; Le Marec, et al., 2001; Li, et al., 2009; McDermott, et al., 2003; Pearlman, 1973; Ravassard, et al., 2009; Ruskin, et al., 2006; Silvestri, 2005; C. Smith & Rose, 1996, 1997; C. T. Smith, et al., 1998; Wang, et al., 2009; Youngblood, et al., 1997). The inverted flowerpot technique is widely variable. An example of a common difference in this technique includes varying the number of platforms available to the rats between one (Davis, et al., 2003; Fu, et al., 2007; Kim, et al., 2005; McDermott, et al., 2003; Ruskin, et al., 2006; Silvestri, 2005; C.

Smith & Rose, 1996, 1997; C. T. Smith, et al., 1998; Youngblood, et al., 1997), three (Bjorness, et al., 2005; Ravassard, et al., 2009) or fourteen (Li, et al., 2009; Wang, et al., 2009).

The number of platforms within the chamber impacts stress levels, where a single platform is considered to be more stressful than multiple platforms, since a single platform results in movement restriction (van Hulzen & Coenen, 1981). The potential for increased stress levels as a result of the number of platforms within the deprivation chamber is a matter of concern when studying the impact of RD on learning, as increased stress levels can also result in impaired learning (Bodnoff, et al., 1995; Conrad, et al., 1996; Foy, Stanton, Levine, & Thompson, 1987; Krugers, et al., 1997; McLay, Freeman, & Zadina, 1998). Though adrenalectomized rats continued to show deficits in learning following RD. (Ruskin, et al., 2006), stress could still act to exaggerate the RD-associated learning deficits and should be controlled to isolate the RD effects on learning from the stress effects.

Another difference in the inverted flowerpot technique used between RD and learning studies is the level of water contained in the deprivation chambers. The level of water is widely variable at 1 - 3 cm from top of the flowerpot platform (Davis, et al., 2003; Fu, et al., 2007; Kim, et al., 2005; Li, et al., 2009; McDermott, et al., 2003; Ruskin, et al., 2006; Silvestri, 2005; Wang, et al., 2009; Youngblood, et al., 1997) or 2 - 3 cm total at the bottom of the chamber (Bjorness, et al., 2005;

Ravassard, et al., 2009). To date, no assessment has been made as to whether the water level during deprivation may alter performance or the extent and specificity of the RS deprivation itself. As several research teams are using differing levels of water within the deprivation chambers, it is necessary to know how this may affect outcome measures in order to compare results across studies.

In a comparison of LTP studies, where a high level of water was used with a single inverted flowerpot (Kim, et al., 2005) as opposed to multiple flowerpots with a low level of water (Ravassard, et al., 2009) within the deprivation chamber, the high water / single platform technique led to longer impairments in LTP. Though the impact of these technique differences have not yet been studied using behavioral tasks, based on these LTP studies, I would predict that behavioral performance variability on spatial learning tasks would similarly be altered.

Specific Aims

The aim of my dissertation is to further our understanding of the interrelationship between sleep and learning. Specifically, using a rat model I address if rRS affects reversal of spatial learning in the Morris water maze in the rat model; and if rRS during initial spatial learning affects subsequent reversal learning. As described earlier, non-spatial extinction learning was impaired when rRS was administered during extinction training and immediately following conditioning,

prior to extinction training (e.g. Fu et al., 2007; Silvestri, 2005). Further it has been previously shown that rRS during initial spatial learning impairs performance (Smith and Rose, 1996; Smith and Rose, 1997). I hypothesized that REM sleep facilitates both initial spatial learning and reversal of spatial learning.

My dissertation investigates the hypothesis that short bouts of REM sleep deprivation impair both initial spatial learning and reversal learning. My first experiment tests the effect of rRS on concurrent initial spatial learning, concurrent reversal learning and subsequent reversal learning using 12 training trials per day in the Morris water maze (Chapter 2). My second experiment in the same training protocol tests how the level of water within the deprivation chambers may alter the rRS effects on performance (Chapter 3). My third experiment tests the effect of rRS on concurrent initial spatial learning, concurrent reversal learning and subsequent reversal learning using 4 training trials per day in the Morris water maze (Chapter 4). Comparing both my first and third experiments (Chapters 2 and 4), I was able to assess the relationship between rRS and learning load for both initial spatial learning and reversal learning. The results from my studies may strengthen our understanding of the role of RS and learning, and shed light on whether the varying results previously described arise from the differing RD chamber designs used. Moreover my research may lend encouragement to a generalized role of RS for learning across spatial tasks, or identify the need to consider each aspect of spatial learning as a unique entity when isolating the role of RS.

References

Aston-Jones, G., & Bloom, F. E. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J Neurosci, 1*(8), 876-886.

Barnes, C. A. (1988). Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. *Trends Neurosci, 11*(4), 163-169.

Beaulieu, I., & Godbout, R. (2000). Spatial learning on the Morris Water Maze Test after a short-term paradoxical sleep deprivation in the rat. *Brain Cogn, 43*(1-3), 27-31.

Bergmann, B. M., Kushida, C. A., Everson, C. A., Gilliland, M. A., Obermeyer, W., & Rechtschaffen, A. (1989). Sleep deprivation in the rat: II. Methodology. *Sleep, 12*(1), 5-12.

Bjorness, T. E., Riley, B. T., Tysor, M. K., & Poe, G. R. (2005). REM restriction persistently alters strategy used to solve a spatial task. *Learn Mem, 12*(3), 352-359.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol, 232*(2), 331-356.

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., & Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J Neurosci, 15*(1 Pt 1), 61-69.

Bramham, C. R., Maho, C., & Laroche, S. (1994). Suppression of long-term potentiation induction during alert wakefulness but not during 'enhanced' REM sleep after avoidance learning. *Neuroscience, 59*(3), 501-509.

Bramham, C. R., & Srebro, B. (1989). Synaptic plasticity in the hippocampus is modulated by behavioral state. *Brain Res, 493*(1), 74-86.

Cirulli, F., Berry, A., & Alleva, E. (2000). Intracerebroventricular administration of brain-derived neurotrophic factor in adult rats affects analgesia and spontaneous behaviour but not memory recall in a Morris Water Maze task. *Neurosci Lett, 287*(3), 207-210.

Cirulli, F., Berry, A., Chiarotti, F., & Alleva, E. (2004). Intrahippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze. *Hippocampus, 14*(7), 802-807.

Cole, A. J., Saffen, D. W., Baraban, J. M., & Worley, P. F. (1989). Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature, 340*(6233), 474-476.

Conrad, C. D., Galea, L. A., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. *Behav Neurosci, 110*(6), 1321-1334.

Crick, F., & Mitchison, G. (1983). The function of dream sleep. *Nature, 304*(5922), 111-114.
Davis, C. J., Harding, J. W., & Wright, J. W. (2003). REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus. *Brain Res, 973*(2), 293-297.
de Bruin, J. P., Sanchez-Santed, F., Heinsbroek, R. P., Donker, A., & Postmes, P. (1994). A behavioural analysis of rats with damage to the medial prefrontal cortex using the Morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. *Brain Res, 652*(2), 323-333.
Fishbein, W., Kastaniotis, C., & Chattman, D. (1974). Paradoxical sleep: prolonged augmentation following learning. *Brain Res, 79*(1), 61-75.
Fogel, S. M., Smith, C. T., & Cote, K. A. (2007). Dissociable learning-dependent changes in REM and non-REM sleep in declarative and procedural memory systems. *Behav Brain Res, 180*(1), 48-61.
Foy, M. R., Stanton, M. E., Levine, S., & Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behav Neural Biol, 48*(1), 138-149.
Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., et al. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience, 144*(4), 1186-1192.
Gaarder, K. (1966). A conceptual model of sleep. *Arch Gen Psychiatry, 14*(3), 253-260.
Gallagher, M., Burwell, R., & Burchinal, M. (1993). Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci, 107*(4), 618-626.
Genzel, L., Dresler, M., Wehrle, R., Grozinger, M., & Steiger, A. (2009). Slow wave sleep and REM sleep awakenings do not affect sleep dependent memory consolidation. *Sleep, 32*(3), 302-310.
Guzowski, J. F., Setlow, B., Wagner, E. K., & McGaugh, J. L. (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. *J Neurosci, 21*(14), 5089-5098.
Harrell, L. E., Barlow, T. S., Miller, M., Haring, J. H., & Davis, J. N. (1984). Facilitated reversal learning of a spatial-memory task by medial septal injections of 6-hydroxydopamine. *Exp Neurol, 85*(1), 69-77.
Hicks, R. A., Okuda, A., & Thomsen, D. (1977). Depriving rats of REM sleep: the identification of a methodological problem. *Am J Psychol, 90*(1), 95-102.
Hobson, J. A., & Pace-Schott, E. F. (2002). The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nat Rev Neurosci, 3*(9), 679-693.
Hodges, H. (1996). Maze procedures: the radial-arm and water maze compared. *Brain Res Cogn Brain Res, 3*(3-4), 167-181.

Hornung, O. P., Regen, F., Danker-Hopfe, H., Schredl, M., & Heuser, I. (2007). The relationship between REM sleep and memory consolidation in old age and effects of cholinergic medication. *Biol Psychiatry, 61*(6), 750-757.

Ishikawa, A., Kanayama, Y., Matsumura, H., Tsuchimochi, H., Ishida, Y., & Nakamura, S. (2006). Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus. *Eur J Neurosci, 24*(1), 243-248.

Iwakiri, H., Matsuyama, K., & Mori, S. (1993). Extracellular levels of serotonin in the medial pontine reticular formation in relation to sleep-wake cycle in cats: a microdialysis study. *Neurosci Res, 18*(2), 157-170.

Jasper, H. H., & Tessier, J. (1971). Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. *Science, 172*(983), 601-602.

Kim, E. Y., Mahmoud, G. S., & Grover, L. M. (2005). REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus. *Neurosci Lett, 388*(3), 163-167.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*(5057), 675-677.

Kimble, D. P. (1968). Hippocampus and internal inhibition. *Psychol Bull, 70*(5), 285-295.

Krugers, H. J., Douma, B. R., Andringa, G., Bohus, B., Korf, J., & Luiten, P. G. (1997). Exposure to chronic psychosocial stress and corticosterone in the rat: effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity. *Hippocampus, 7*(4), 427-436.

Le Marec, N., Beaulieu, I., & Godbout, R. (2001). Four hours of paradoxical sleep deprivation impairs alternation performance in a water maze in the rat. *Brain Cogn, 46*(1-2), 195-197.

Li, S., Tian, Y., Ding, Y., Jin, X., Yan, C., & Shen, X. (2009). The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats. *Learn Behav, 37*(3), 246-253.

Louie, K., & Wilson, M. A. (2001). Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron, 29*(1), 145-156.

Maei, H. R., Zaslavsky, K., Teixeira, C. M., & Frankland, P. W. (2009). What is the Most Sensitive Measure of Water Maze Probe Test Performance? *Front Integr Neurosci, 3*, 4.

Mavanji, V., & Datta, S. (2003). Activation of the phasic pontine-wave generator enhances improvement of learning performance: a mechanism for sleep-dependent plasticity. *Eur J Neurosci, 17*(2), 359-370.

McDermott, C. M., Hardy, M. N., Bazan, N. G., & Magee, J. C. (2006). Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus. *J Physiol, 570*(Pt 3), 553-565.

McDermott, C. M., LaHoste, G. J., Chen, C., Musto, A., Bazan, N. G., & Magee, J. C. (2003). Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *J Neurosci, 23*(29), 9687-9695.

McGrath, M. J., & Cohen, D. B. (1978). REM sleep facilitation of adaptive waking behavior: a review of the literature. *Psychol Bull, 85*(1), 24-57.
McLay, R. N., Freeman, S. M., & Zadina, J. E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. *Physiol Behav, 63*(5), 933-937.
Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. *Neurosci Lett, 163*(1), 109-113.
Newman, E. A., & Evans, C. R. (1965). Human dream processes as analogous to computer programme clearance. *Nature, 206*(983), 534.
Park, S. P., Lopez-Rodriguez, F., Wilson, C. L., Maidment, N., Matsumoto, Y., & Engel, J., Jr. (1999). In vivo microdialysis measures of extracellular serotonin in the rat hippocampus during sleep-wakefulness. *Brain Res, 833*(2), 291-296.
Pearlman, C. (1973). REM sleep deprivation impairs latent extinction in rats. *Physiol Behav, 11*(2), 233-237.
Penalva, R. G., Lancel, M., Flachskamm, C., Reul, J. M., Holsboer, F., & Linthorst, A. C. (2003). Effect of sleep and sleep deprivation on serotonergic neurotransmission in the hippocampus: a combined in vivo microdialysis/EEG study in rats. *Eur J Neurosci, 17*(9), 1896-1906.
Poe, G. R., Nitz, D. A., McNaughton, B. L., & Barnes, C. A. (2000). Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. *Brain Res, 855*(1), 176-180.
Portas, C. M., & McCarley, R. W. (1994). Behavioral state-related changes of extracellular serotonin concentration in the dorsal raphe nucleus: a microdialysis study in the freely moving cat. *Brain Res, 648*(2), 306-312.
Portell-Cortes, I., Marti-Nicolovius, M., Segura-Torres, P., & Morgado-Bernal, I. (1989). Correlations between paradoxical sleep and shuttle-box conditioning in rats. *Behav Neurosci, 103*(5), 984-990.
Pouzet, B., Welzl, H., Gubler, M. K., Broersen, L., Veenman, C. L., Feldon, J., et al. (1999). The effects of NMDA-induced retrohippocampal lesions on performance of four spatial memory tasks known to be sensitive to hippocampal damage in the rat. *Eur J Neurosci, 11*(1), 123-140.
Rauchs, G., Desgranges, B., Foret, J., & Eustache, F. (2005). The relationships between memory systems and sleep stages. *J Sleep Res, 14*(2), 123-140.
Ravassard, P., Pachoud, B., Comte, J. C., Mejia-Perez, C., Scote-Blachon, C., Gay, N., et al. (2009). Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. *Sleep, 32*(2), 227-240.
Regnard, M. (1881). Sleep and Somnambulisnm. *Science, 2*(50), 258-262.
Ribeiro, S., Mello, C. V., Velho, T., Gardner, T. J., Jarvis, E. D., & Pavlides, C. (2002). Induction of hippocampal long-term potentiation during waking leads to increased extrahippocampal zif-268 expression during ensuing rapid-eye-movement sleep. *J Neurosci, 22*(24), 10914-10923.

Romcy-Pereira, R., & Pavlides, C. (2004). Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP. *Eur J Neurosci, 20*(12), 3453-3462.
Ruskin, D. N., Dunn, K. E., Billiot, I., Bazan, N. G., & LaHoste, G. J. (2006). Eliminating the adrenal stress response does not affect sleep deprivation-induced acquisition deficits in the water maze. *Life Sci, 78*(24), 2833-2838.
Shouse, M. N., Staba, R. J., Saquib, S. F., & Farber, P. R. (2000). Monoamines and sleep: microdialysis findings in pons and amygdala. *Brain Res, 860*(1-2), 181-189.
Siegel, J. M. (2001). The REM sleep-memory consolidation hypothesis. *Science, 294*(5544), 1058-1063.
Silvestri, A. J. (2005). REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiol Behav, 84*(3), 343-349.
Silvestri, A. J., & Root, D. H. (2008). Effects of REM deprivation and an NMDA agonist on the extinction of conditioned fear. *Physiol Behav, 93*(1-2), 274-281.
Smith, C. (1985). Sleep states and learning: a review of the animal literature. *Neurosci Biobehav Rev, 9*(2), 157-168.
Smith, C. (1995). Sleep states and memory processes. *Behav Brain Res, 69*(1-2), 137-145.
Smith, C., & Butler, S. (1982). Paradoxical sleep at selective times following training is necessary for learning. *Physiol Behav, 29*(3), 469-473.
Smith, C., & Lapp, L. (1986). Prolonged increases in both PS and number of REMS following a shuttle avoidance task. *Physiol Behav, 36*(6), 1053-1057.
Smith, C., & Rose, G. M. (1996). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiol Behav, 59*(1), 93-97.
Smith, C., & Rose, G. M. (1997). Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze. *Behav Neurosci, 111*(6), 1197-1204.
Smith, C., & Wong, P. T. (1991). Paradoxical sleep increases predict successful learning in a complex operant task. *Behav Neurosci, 105*(2), 282-288.
Smith, C., Young, J., & Young, W. (1980). Prolonged increases in paradoxical sleep during and after avoidance-task acquisition. *Sleep, 3*(1), 67-81.
Smith, C. T., Conway, J. M., & Rose, G. M. (1998). Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. *Neurobiol Learn Mem, 69*(2), 211-217.
Stickgold, R., & Walker, M. P. (2005). Sleep and memory: the ongoing debate. *Sleep, 28*(10), 1225-1227.
van Hulzen, Z. J., & Coenen, A. M. (1981). Paradoxical sleep deprivation and locomotor activity in rats. *Physiol Behav, 27*(4), 741-744.
Vazquez, J., & Baghdoyan, H. A. (2001). Basal forebrain acetylcholine release during REM sleep is significantly greater than during waking. *Am J Physiol Regul Integr Comp Physiol, 280*(2), R598-601.

Venkatakrishna-Bhatt, H., Bures, J., & Buresova, O. (1979). Paradoxical sleep deprivation retards extinction of conditioned taste aversion. *Behav Neural Biol, 25*(1), 133-137.

Vertes, R. P. (2004). Memory consolidation in sleep; dream or reality. *Neuron, 44*(1), 135-148.

Vertes, R. P., & Eastman, K. E. (2000). The case against memory consolidation in REM sleep. *Behav Brain Sci, 23*(6), 867-876; discussion 904-1121.

Vertes, R. P., & Siegel, J. M. (2005). Time for the sleep community to take a critical look at the purported role of sleep in memory processing. *Sleep, 28*(10), 1228-1229; discussion 1230-1223.

Walker, M. P., & van der Helm, E. (2009). Overnight therapy? The role of sleep in emotional brain processing. *Psychol Bull, 135*(5), 731-748.

Wang, G. P., Huang, L. Q., Wu, H. J., Zhang, L., You, Z. D., & Zhao, Z. X. (2009). Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation. *Neuroreport, 20*(13), 1172-1176.

Whishaw, I. Q., & Tomie, J. (1997). Perseveration on place reversals in spatial swimming pool tasks: further evidence for place learning in hippocampal rats. *Hippocampus, 7*(4), 361-370.

Whishaw, I. Q. (1998). Place learning in hippocampal rats and the path integration hypothesis. *Neurosci Biobehav Rev, 22*(2), 209-220.

Yoo, S. S., Hu, P. T., Gujar, N., Jolesz, F. A., & Walker, M. P. (2007). A deficit in the ability to form new human memories without sleep. *Nat Neurosci, 10*(3), 385-392.

Youngblood, B. D., Zhou, J., Smagin, G. N., Ryan, D. H., & Harris, R. B. (1997). Sleep deprivation by the "flower pot" technique and spatial reference memory. *Physiol Behav, 61*(2), 249-256.

Chapter 2

REM sleep deprivation during learning disrupts subsequent reversal learning

Abstract

Some disagreement in the literature surrounds whether rapid eye movement (REM) sleep deprivation or restriction impairs declarative memory. One aspect that has not been studied is how REM sleep restriction affects either a subsequent reversal learning task or reversal learning concurrent to REM sleep restriction. Using both a classical allocentric training protocol and a reversal training paradigm in the Morris water maze, animals were trained with 12 trials per day followed by 6 hrs of REM sleep deprivation using the inverted flowerpot technique. I tested whether REM sleep restriction affects initial spatial learning, subsequent reversal learning and concurrent reversal learning. Two experiments were performed. Experiment 1 focused on the effects of REM sleep restriction on concurrent initial spatial learning and subsequent reversal learning (n = 17).

Experiment 2 focused on the effects of REM sleep restriction on concurrent reversal learning (n = 24). The stress of REM sleep restriction was controlled for by the addition of a REM sleep deprivation group who were deprived later, outside the reported REM sleep sensitive learning window. I found that REM sleep restriction does not significantly affect concurrent spatial or reversal learning compared with controls or later REM sleep deprived animals. However, REM sleep restriction during initial learning was associated with a deficit in subsequent reversal learning. Prior REM sleep restriction seems to reduce the flexibility of subsequent learning. This is the first study to report on the effects of REM sleep restriction on either concurrent reversal learning or subsequent learning in the Morris water maze.

Introduction

While there is no universal consensus on the exact relationship between REM sleep (RS) and learning, a number of findings suggest that RS is tightly linked with specific types of learning, such as spatial learning, which is generally thought to rely on the hippocampus. Increases in RS have been described following learning in a number of studies (e.g. Fishbein, Kastaniotis et al. 1974; Smith, Young et al. 1980; Smith and Butler 1982; Smith and Lapp 1986; Portell-Cortes, Marti-Nicolovius et al. 1989; Smith and Wong 1991; Bramham, Maho et al. 1994; Smith and Rose 1997; Mavanji and Datta 2003). Increases in RS can be predictive of performance improvements the following day (Smith and Wong 1991), which suggests a functional relationship between RS and learning.

To determine if learning is affected by RS, many studies have relied on REM sleep deprivation (RD) or RS restriction (rRS, short periods of RD) prior to or following learning. In the rat animal model, RD associated deficits have been described for spatial learning tasks, thought to be dependent on the hippocampus, using the Morris water maze (e.g. Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Ruskin, Dunn et al. 2006; Li, Tian et al. 2009; Wang, Huang et al. 2009), the radial arm maze (Smith, Conway et al. 1998) and the Poe 8-box maze (Bjorness, Riley et al. 2005).

Another learning paradigm that has been suggested to be hippocampus dependent is reversal learning of spatial tasks. Reversal of spatial learning is the learning of a new response (e.g. movement to a new target location) when in a familiar environment or presented with the same environmental stimuli. Hippocampal-dependent reversal learning can be studied using a reversal learning paradigm in both the Morris water maze (e.g. Morris, Hagan et al. 1986; Conrad and Roy 1993; Whishaw and Tomie 1997; Blokland, de Vente et al. 1999; Hoh, Beiko et al. 1999; Pouzet, Welzl et al. 1999; Cirulli, Berry et al. 2000; Guzowski, Setlow et al. 2001; Joyal, Strazielle et al. 2001; Lacroix, White et al. 2002; Sullivan and Gratton 2002; Cirulli, Berry et al. 2004; Cimadevilla and Arias 2008) and the 8-arm maze task (e.g. Conrad and Roy 1993; Pouzet, Welzl et al. 1999). Specifically, hippocampal damage or an alteration in hippocampal activity can lead to disruption of reversal learning (Morris, Hagan et al. 1986; Whishaw

and Tomie 1997). These studies suggest that reversal learning is also mediated through the hippocampus, and that reversal learning may have greater measurable changes in response to altered hippocampal activity than initial spatial learning (Pouzet, Welzl et al. 1999; Cirulli, Berry et al. 2000; Cirulli, Berry et al. 2004).

While the effects of RD on the reversal of spatial learning have not been studied, the effects on conditioned extinction have been (e.g. Pearlman 1973; Silvestri 2005; Fu, Li et al. 2007). Though extinction learning is the uncoupling between a stimulus and response, extinction learning can require similar neural mechanisms and brain regions as reversal learning (e.g. van der Meulen, Bilbija, Joosten, de Bruin, & Feenstra, 2003). Typically, it has been shown that both concurrent conditioning and concurrent extinction are impaired in the rat model (e.g. Pearlman 1973; Fu, Li et al. 2007). However, in a recent study on honeybees, consolidation was unaffected by sleep deprivation, while extinction consolidation was impaired (Hussaini et al., *in press*). Further, experiments on the effects of rRS or RD on subsequent extinction have shown impairments for both the extinction of a conditioned bar pressing task (Pearlman 1973) and the extinction of cued fear conditioning (Silvestri 2005) in rodents. Though we do not know the effects of rRS or RD on concurrent reversal of spatial learning or subsequent reversal learning, based on these conditioning and extinction studies, it could be predicted that performance deficits would be observed.

It is widely accepted that RS is associated with a drop in hippocampal norepinephrine (NE). In a study of NE depletion and reversal learning, it was shown that the lack of NE was associated with enhanced reversal learning in the 8-arm maze (Harrell, Barlow et al. 1984). This indicates that RS could facilitate reversal learning. Based on this finding and the typically seen RD or rRS-associated deficits in spatial learning (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Smith, Conway et al. 1998; Bjorness, Riley et al. 2005; Ruskin, Dunn et al. 2006; Li, Tian et al. 2009; Wang, Huang et al. 2009) and impaired hippocampal longer-term potentiation (LTP, Davis, Harding et al. 2003; Romcy-Pereira and Pavlides 2004; Kim, Mahmoud et al. 2005; Ishikawa, Kanayama et al. 2006; McDermott, Hardy et al. 2006; Ravassard, Pachoud et al. 2009), which is a mechanism of learning, I predicted that rRS concurrent with reversal learning would result in clear performance deficits. To date, no studies have been published on the effect of rRS on either concurrent or subsequent reversal learning in the Morris water maze.

Previous studies have described the presence of RD sensitive windows for learning (e.g. Pearlman 1973; Leconte, Hennevin et al. 1974; Smith and Rose 1996; Smith and Rose 1997; Smith, Conway et al. 1998; Silvestri 2005; Fu, Li et al. 2007). The timing of these RD sensitive windows appear to be independent of task (Pearlman 1973; Leconte, Hennevin et al. 1974; Smith and Rose 1997; Smith, Conway et al. 1998; Silvestri 2005; Fu, Li et al. 2007), but dependent on learning load (Smith and Rose 1996; Smith and Rose 1997) where the time-

dependent RD sensitive window for 12 trials of training in the Morris water maze is immediately following training, while 4 trials in the Morris water maze leads to a sensitive window starting 5 hrs after training. Based on these findings, my target rRS was immediately following training. In experiment 2 I included a 6 hr delayed rRS group as a control for rRS. I **hypothesized** that 6 hrs of RD immediately following reversal learning results in concurrent performance deficits, with no affect on animals that received the delayed 6 hrs RD. I **hypothesized** that 6 hrs of RD immediately following initial spatial learning results in concurrent performance deficits similar to those reported before. Lastly, I **hypothesized** that rRS during initial spatial learning results in performance deficits during subsequent reversal learning.

Many previous studies on the effects of RD in the Morris water maze have reported on either latency to platform (Smith and Rose 1996; Smith and Rose 1997; Li, Tian et al. 2009; Wang, Huang et al. 2009), pathlength (Li, Tian et al. 2009; Wang, Huang et al. 2009) number of target quadrant entries during training (Smith and Rose 1996), area under the curve for both latency and pathlength (Youngblood, Zhou et al. 1997) or percent time spent in target quadrant during a probe trial (Wang, Huang et al. 2009). My current study is the first study to report on the effects of rRS on Morris water maze learning using the more sensitive Gallagher measures (Gallagher, Burwell et al. 1993). The difficulty in interpreting latency measures is that it is impossible to tell whether the platform was found by chance by using non-spatial strategies or purposefully using spatial mapping

(Gallagher, Burwell et al. 1993; discussed in Hodges 1996). Similarly, pathlength is also difficult to interpret. The Gallagher cumulative distance from the target platform measure is less vulnerable to spatial independent search strategies (Gallagher, Burwell et al. 1993) during training. Similarly, the Gallagher average proximity to platform measure is the most sensitive to differences in search pattern (Maei, Zaslavsky et al. 2009) during a probe trial. My study is one of the first in-depth reports on rRS following the Morris water maze to look at a wider range of variables during both training and the probe trials.

Methods

Rats were tested for the effect of REM sleep deprivation administered during reversal learning (concurrent reversal learning), during initial spatial learning (concurrent initial spatial learning) and reversal learning when the rats no longer had disrupted REM sleep (subsequent reversal learning).

Animals

All rats used in this study were Sprague-Dawley male rats (~380 g; Harlan Indianapolis, IN). Animals were housed in a 12:12 light cycle at an average temperature of 23 °C. Procedures were approved by the animal review board, the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. Rats had *ad libitum* access to fresh drinking water and food at all

times except while in the water maze. Each rat was weighed at the start of each experimental day, before testing, to monitor changes in percent body weight.

REM deprivation protocol

The REM deprivation tank (61 x 47 x 50.8 cm; Figure 2.1) contained three inverted flowerpots (24 cm tall), forming 3 bases for the rats to sit on (Bjorness, Riley et al. 2005; Ravassard, Pachoud et al. 2009). Each base was 6 cm in diameter to maintain the necessary rat weight-to-flowerpot base size ratio previously shown to induce REM deprivation (Hicks, Okuda et al. 1977; McGrath and Cohen 1978). The distance between the platforms was 9 cm to allow the rats to easily move between them. Drinking water and food were freely available in the deprivation chamber. A netted lid was placed over the REM deprivation tanks, leaving enough space for the rats to rear up without reaching the lid. The netting enabled the experimenter to observe the rats remotely using an overhead camera projected to a neighboring room. Room temperature was kept constant at 23 °C. A low level of water (2 cm in height) in the base of the deprivation tank was used for this study. This level of water prevents the rats' tails from dangling in the water, which would reduce their ability to thermoregulate. Rats were closely observed for signs of distress and to monitor their behavior.

Visual Water Maze protocol

Initially, rats were tested for visual and motor acuity using a visual platform in the water maze (Morris, 1984) to ensure that rats selected for the spatial Morris

water maze would have the ability to perform the task both for the visual and motoric components. The water maze consisted of a circular tank (170 cm diameter), painted black and filled with clear water. The platform (14 cm diameter, 26.7 cm tall) was covered with a striped white and navy pattern. The top of the platform was 2 cm above the surface of the water. The water temperature was maintained at ~ 27 °C. The tank was surrounded by a black curtain to remove all spatial cues, and was well lit with overhead lighting. During testing, each rat was placed in an individual water maze cage, consisting of a towel-lined cage with a microfilter lid. After 10 mins acclimation to the room, each rat was placed, in turn, into the tank at one of four locations (North, South, East or West) and allowed a maximum of 60 seconds to find the platform. If the rat did not find the visible platform within the time limit, it was guided to the platform location. Once on the platform, each rat remained there for an additional 20 seconds. Each rat in the testing group completed its trial in turn, before the next trial was begun. At the start of each trial, the platform was moved to one of four different locations (Northeast, Northwest, Southeast or Southwest). Each rat received a total of 5 trials per day for two consecutive days. At the end of the second day, the 12 rats with the lowest average latency to the visual platform were selected to continue for the spatial learning component of the experiment. Their performance on the visual platform task indicated that they had both sufficient vision and the motor and mental competence to perform the task. Each rat was then placed into individual housing (plexiglas cages 45.7 x 24.1 x 20.3 cm). Rats were allowed 5 days to acclimate to their environment. During these 5

days, rats were placed into individual REM sleep deprivation chambers for 45 minutes each on 2 days (see REM deprivation protocol, above, for further description).

Experiment 1

Morris Water Maze protocol

Rats were randomly assigned into one of 3 groups: Controls (CONR; n = 8), delayed REM sleep deprivation (rRSRev6-12; n = 8) and immediate REM sleep deprivation (rRSRev0-6; n = 8). Though both RD periods lasted the same duration of time, the delayed group was used as a rRS control based on Smith and Rose's (1997) work on RS windows. The same water maze tank (Figure 2.2) and pedestal as described in the visual platform protocol, were used, however the platform was covered with black material. Unlike the visual platform, the standard Morris water maze has a hidden platform, where the top of the platform is 14 mm below the surface of the water. The room contained a number of spatial markers (e.g. large black curtain in one corner; large picture on one side; rack with hoses and mops on another side). Latency to platform was measured using a hand stopwatch, while visual tracking data were acquired using 4.1 EthoVision XT (Noldus Information Technology b.v., Netherlands). Data were acquired at a sampling rate of 15 Hz and was later down-sampled offline.

Within 30 mins of lights-on, rats were weighed and put into their individual water maze cages for the water maze session. At the start of day 1, each rat was

placed, in turn, onto the hidden platform for 20 seconds to introduce the hidden platform. At the start of each trial, a rat was placed into the tank at one of four entry points, North, South, East or West. The entry point for each trial was constant across rats and semi-randomized across trials. No trial had the same entry point as the prior trial, but on any given trial number, all rats had the same entry point. Maximum trial length was set at 90 seconds. If a rat did not find the platform within the allowed time they were guided to the hidden platform. After each trial the rat remained on the hidden platform for an additional 20 seconds. All rats were run in groups of 6, where the whole group completed each trial before the next trial was begun. In total, 12 training trials were run each day, with an additional probe trial at the start of day 4 and day 6 (see Figure 2.3 for the protocol outline).

For the two probe trials, the hidden platform was removed and rats were placed into the water maze tank for 60 seconds. At the end of the 60 seconds they were rescued and returned to their water maze cages.

For the Learning Phase of this experiment on days 1 to 4, the hidden platform was located in the Northeast quadrant, 38 cm from the tank wall, equidistant from both the North and the East edge of the quadrant.

The Reversal Phase started on the 7th trial of day 4, at which point the platform was placed in the opposite quadrant of the tank (Southwest quadrant). All room

cues remained in their original positions, not changing between the Learning Phase and the Reversal Phase. The platform remained in this location for both day 5 and day 6.

After the 12th trial on each day, rats remained in their maze cages for 10 minutes to dry and then were returned to either their homecage or to the REM deprivation tanks. All RD periods lasted for 6 hrs. The rRSRev0-6 group was RS deprived for the first 6 hrs immediately following training on Days 4 and 5. The rRSRev6-12 group was RS deprived for 6 hrs starting 6 hrs after learning, therefore in the 6-12 hr postlearning window.

The dependent variables measured during learning trials were latency, path length, velocity and Gallagher cumulative distance from the platform (Gallagher, Burwell et al. 1993). The latter variable is the distance of the rat from the target platform at each second. During probe trials, the dependent variables were the Gallagher average proximity error to the platform, number of platform crossings, percent time in target quadrant, path length and velocity. All measures other than latency were acquired and processed using EthoVision XT. Off-line, data acquired using EthoVision XT were interpolated to fill in any missing data points. Velocity was interpolated by the average of prior and post samples. Distance was determined by the duration of time and the relevant interpolated velocity. For the Gallagher and 'in zone' measures (used to calculate percent time in quadrant and number of platform crossings), default EthoVision interpolation was retained,

then downsampled to 1 Hz. When calculating the Gallagher measures (average proximity to the platform and cumulative distance from the platform) the time taken to swim directly between the initial start location and platform location for each individual rat was not corrected for in any of the trials. All other measures were downsampled to 5 Hz.

Data were analyzed as trialsets (average performance across 3 consecutive trials: Trials 1 - 3, 4 - 6, 7 - 9, 10 – 12) and in specific cases as single trials. Retention was measured by comparing the last trial of a day with the first trial of the subsequent day. Retention was also calculated for trialset 4 (trials 10-12) vs. trialset 1 (trials 1-3) the subsequent day. Comparisons were not made for retention differences on Days 4 and 6 due to potential interference resulting from the probe trial. Specifically, for the first trial after the probe the rats may tend to search more areas rather than go straight to the old platform location because they already discovered that the location was empty in the probe trial.

In order to determine if there were initial differences at the start of each trial, the first 5 s of data for the Gallagher cumulative error were analyzed separately. Using this measure, it was possible to determine if, at the start of the trial, rats tended towards the platform location. I chose a 5 s initial period to allow rats to swim away from the wall and start their chosen path. To determine initial differences for the 60 s probe trials, the first 10 s were analyzed separately for all measures. Ten, rather than 5 seconds were analyzed in the probes to allow the

rats more time to search and show location preference. I could not allow the full 10 s during the training trials because the rats often found the platform within that time period. The initial differences during the probe trial are particularly important as later measures may reflect the rat's decision to change their search patterns after not finding the platform in the expected position rather than spend the entire probe trial searching in that location. Therefore, the first 10 s can provide information on the level of learning of the rat, where the entire 60 s probe can speak to the persistence of the rat to search for the prior platform location.

Experiment 2

To determine if the effects of RD during the initial spatial learning had an effect on subsequent reversal learning, seventeen rats were split into a control group (CONL; n = 7) or a group who were RS deprived during the initial spatial learning (rRSL; n = 10). The protocol and procedures were identical as in Experiment 1, however the RD period for rRSL was given immediately following learning for 6 hrs on Days 1, 2 and 3. On Days 4 and 5, all rats were immediately returned to their homecages (see Figure 2.4). The data analyses were also similar except that for my measure of the first 5 s of cumulative distance, as there was a group difference on the last trialset of Day 1 that could erroneously contribute to or mask manipulation-related differences, I normalized performance across the study to Day 1 within each trialset. Therefore Day 2 trialset 1 was normalized to Day 1 trialset 1. Similarly, Day 2 trialset 2 was normalized to Day 1 trialset 2 and so forth.

Statistics

All analyses were done using SPSS (SPSS Inc. Chicago, IL). In all cases, when sphericity could not be assumed during a Repeated Measures Analyses Of Variance (RMANOVA), the Huynh-Feldt correction was used.

RMANOVA were used, and post-hoc analyses using a Tukey correction were administered when an effect was found. The Reversal Phase was analyzed with 4 trialsets per day for 2 days (Days 5 and 6). To determine differences within each day, RMANOVA were used across the 4 trialsets on Days 5 and 6, and across the latter 2 trialsets on Day 4. Retention at the start of Day 5 was analyzed using a one-way ANOVA on the difference between the last trialset on Day 4 and the first trialset on Day 5. This analysis was also done for a single trial (the first trial on Day 5 vs. the last trial on Day 4) instead of the trialset.

The Learning Phase was analyzed with 4 trialsets per day for 3 days (Days 1, 2 and 3). To determine differences within each day, RMANOVA were used across the 4 trialsets on Days 1, 2 and 3, and across the first 2 trialsets on Day 4. Retention at the start of Days 2 and 3 was analyzed using a one-way ANOVA, similar to the analysis used on the Reversal Phase.

The level of learning was determined by variables measured during the probe trials, which were tested using one–way ANOVAs to determine group

differences. RMANOVA were used to compare across probe trials, to determine if learning was more pronounced for a measure on one probe trial as compared to the other. RMANOVA were also used to analyze the probe trials, where performance measures in reference to the two target locations on Day 6 (Learning Phase and Reversal Phase platform locations) were compared to determine whether rats showed a search preference for one location over the other. A RMANOVA was also used to analyze rat weights across the experiment.

Summary

In an effort to thoroughly investigate the effects of RD on spatial learning in the Morris water maze, a number of variables were tested. Measures for training trials include: latency to platform, pathlength, velocity, and the Gallagher cumulative distance from platform. Indices of learning on probe trials were: number of platform crossings, the Gallagher average proximity to the platform and percent time spent in target quadrant. In addition, velocity and pathlength were also measured.

This experiment was done to determine if RD during the Reversal Phase resulted in a deficit in performance. Further the experiment was designed to determine if there was a differential effect between immediate and delayed RD following reversal learning. Training during the Reversal Phase was analyzed across days (2 days, 4 trialsets per day), within each day (Days 5, and 6: 4 trialsets; Day 4: 2 trialsets) and within the three trials of specific trialsets.

Results

Experiment 1

My hypothesis for experiment 1 was that 6 hrs of rRS immediately following reversal learning would lead to poorer performance while rRS starting 6 hrs after learning should not lead to any deficits in performance. Instead I found that neither rRS periods resulted in altered performance during reversal learning. My hypothesis for experiment 2 was the 6 hrs of rRS immediately following spatial learning would result in performance deficits. My results do not support this hypothesis, but instead suggests a delayed effect.

Experiment 1 - Training trials during the Reversal Phase

All rats learned to find the hidden platform as seen by improved performance across days (latency, $p = 0.001$) and trialsets ($p < 0.001$, linear fit) and a day x trialset interaction ($p = 0.01$, linear fit) for all measures (Figures 2.5, 2.6 and 2.7). There were no group differences or interactions measured for latency (Figure 2.5), pathlength (Figure 2.6) or cumulative distance from the platform (Figure 2.7). On Day 5 there was a trend for a trialset x group interaction ($p = 0.084$) for pathlength, but no group main effects were found. No other group differences were found for any of these 3 variables across the Reversal Phase training.

The level of retention was assessed to determine if REM deprivation caused a 'resetting' or initial forgetfulness on the following day. To measure this, pathlength, latency and cumulative distance variables for the first trialset on Day 5 were subtracted from the same measure for the last trialset on Day 4. There was a trend for a group difference in retention of latency to platform at the start of Day 5 ($p = 0.057$), where CONR had poorer retention as compared to rRSRev0-6 ($p = 0.046$) (Figure 2.5). There was also a trend for a group difference in the level of retention at the start of Day 5 ($p = 0.074$) as measured by the cumulative distance from the platform, where CONR had poorer retention than rRSRev0-6 ($p = 0.061$) (Figure 2.7). For both latency and cumulative distance, there were no differences in retention when individual trials were tested. No group differences were identified for pathlength. The reset between Day 6 and Day 5 could not be assessed due to potential interference from the probe trial at the start of Day 6.

The first 5 s of each training trial was analyzed to determine if, at the start of the trial, any group took a more direct path to the platform as compared to the other groups, which could be identified using the Gallagher cumulative distance measure. Therefore, this could be considered as a measure of initial preference. I chose to use 5 s based on the duration of the faster trials, to limit the time window to focus on initial swim path, while allowing sufficient time for the rat to start on its swim path. However, no group differences were identified (Figure 2.8).

All groups swam at the same swim speed across the Reversal Phase.

Experiment 1 - Day 4 probe compared to Day 6 probe for platform locations

To determine the level of learning between the two phases, performance on Day 4 probe versus Day 6 probe was compared. Overall, rats performed better on Day 4 than Day 6, spending more time in the target quadrant ($p < 0.001$; Figure 2.9) and had lower average proximity error ($p < 0.001$). Together these data suggest that the Learning Phase platform location on Day 4 probe test was remembered better than the Reversal Phase platform location on Day 6 probe test. This was to be expected, as there were twice as many training trials before the Learning Phase probe as compared to the Reversal Phase probe. No group differences were found on either the Learning Phase or the Reversal Phase probe trials. Further, time in the two platform locations were compared during the Day 6 probe to determine if either group had a preference for one learned platform over the other. Overall all groups preferred the reversal platform location (number of platform crossings, $p = 0.007$, Figure 2.10), and no other group differences were found.

Experiment 1 - First 10 s of probe trials

I also looked at the first 10 s only of the probe trials to determine if initial differences were present between the groups that could have been masked

when the entire probe trial was analyzed. However, no group differences were identified.

Experiment 1 - Summary

All groups improved their performance during the Reversal Phase. Therefore, both 6 hrs of immediate and delayed RD during the Reversal Phase did not impair learning of the reversal platform location. A trend was identified where rRSRev0-6, first experiencing RD at the end of Day 4 training, had *better* retention at the start of Day 5 as compared to CONR (Table 2.1).

Experiment 1 - Percent body weight

Percent body weight was used as an indicator of stress, where decreased percent body weight can be a sign of increased stress levels. When percent body weight was calculated based on the rats' body weights on Day 4, a trend for a group x day interaction was measured ($p = 0.099$) and a significant group main effect ($p = 0.05$) in which rRSRev6-12 tended to have lost relatively more weight than rRSRev0-6 across the study. When percent body weights were calculated based on Day 4, the start of reversal learning, Day 5 had a significant group difference ($p = 0.012$) where both CONR ($p = 0.029$) and rRSRev6-12 ($p = 0.02$) had lost more percent body weight than rRSRev0-6 (Figure 2.11). This was not detected either for Day 6 as a percent of Day 4 or for either Days 5 or 6 when percent body weights were based on Day 1 body weights. The results for body

weight indicated that relative to Day 4, the first day of reversal training and rRS, led to drops in body weight for CONR and rRSRev6-12 that were not seen any of the other days.

Experiment 2 - The effects of RD during initial spatial learning

As there were no significant differences in performance when RD was administered during reversal learning, I sought to determine whether RD during initial learning had a subsequent effect on reversal learning. During the Learning Phase, all rats had performance improvements across the days and trials. There was no effect of RD on performance measures for latency (Figure 2.12), pathlength and cumulative distance (Figure 2.13) during the Learning Phase. When only the first 5 s of the trial was analyzed for differences in cumulative distance (Figure 2.14), both groups also had similar measures. Retention at the start of Days 2 and 3 were analyzed for all 3 performance variables. No differences were identified between the RS deprived during spatial learning group (rRSL) and normal sleeping controls (CONL). On the Day 4 probe trial, no group differences were identified between rRSL and CONL for percent time in target quadrant (Figures 2.15), number of platform crossings, average proximity error (Figures 2.16) or pathlength. Further no group differences were identified either during the Learning Phase probe or training trials. Therefore, similar to my results for rRS during reversal learning, rRS during spatial learning did not significantly impair or enhance performance.

Experiment 2 - The effects of rRSL on subsequent reversal learning

During the Reversal Phase, no group differences were identified for latency (Figure 2.12), pathlength or cumulative distance (Figure 2.13) when the whole trial was considered during training. However, when data were normalized to performance on Day 1, across Day 4 Reversal Phase CONL performed significantly better than rRSL ($p = 0.041$) during the first 5 s of the trials for the cumulative distance measure (Figure 2.14). When the individual trialsets were investigated, it was the second trialset of reversal learning on Day 4 that was significantly different ($p = 0.044$). At the start of Day 5 (trialset 1) CONL continued to perform better than rRSL, swimming closer to the new platform location ($p = 0.032$) during the first 5 s of the trials for cumulative distance. By the second trialset on Day 5, performance was equivalent between both groups. These data suggest during the start of the Reversal Phase, rRSL had greater initial error in path direction with respect to the new platform location.

Retention at the start of Day 5 (trialset 1) as compared to the end of Day 4 was not different for any of the variables (latency, pathlength, cumulative distance) tested.

During the Reversal Phase on Day 4 rRSL swam faster than CONL ($p = 0.025$). This could indicate that rRSL were more stressed than CONL at the start of reversal training.

On the Day 6 probe, there was a group difference in Gallagher's average proximity error to the Reversal Phase platform, in which CONL swam in closer proximity to the platform area than rRSL ($p = 0.027$, Figure 2.16).

Further investigation indicated that rRSL swam in closer proximity to the initial Learning Phase platform location ($p = 0.063$) with a significant platform location x group interaction on Day 6 ($p = 0.015$). Thus those animals that were not allowed to experience early RS after training during the Learning Phase preferred the old platform location whereas those that had sufficient RS preferred the reversal location. No other group differences were identified during the Day 6 probe trial.

Experiment 2 - First 10 s of the Day 6 probe trial

In the first 10 s of the Day 6 probe trial, CONL had significantly more crossings of both the Learning Phase and the Reversal Phase platform locations ($p = 0.046$, Figure 2.17). No significant difference in the number of platform crossings was identified between CONL and rRSL when the Reversal Phase platform alone was measured, but there was a trend for CONL to have more Learning Phase platform crossings ($p = 0.07$) than rRSL. Further CONL tended to spend more time in both the Learning Phase and the Reversal Phase target quadrants than rRSL ($p = 0.067$) during the first 10 s of the Day 6 probe. When either target quadrant was analyzed separately, no group differences were identified. No other group differences were found in average proximity to platform, pathlength or swim speed.

Experiment 2 - Summary

Overall these results indicate that while RD during the Learning Phase did not result in a change in performance during the Learning Phase (Table 2.2 A), it did cause a disruption of subsequent reversal learning (Table 2.2 B). Specifically, at the start of reversal training, rRSL swam faster than CONL. At the start of Day 5, rRSL swam farther away from the Reversal Phase platform than CONL during the first 5 s of the trialset. The Day 6 probe trial indicated that CONL swam significantly closer to the reversal platform than rRSL, while rRSL tended to swim nearer the Learning Phase platform location than CONL. When only the first 10 s of the probe trial were analyzed, CONL had significantly more crossings of either the Learning Phase or Reversal Phase platform location than rRSL.

Experiment 2 - Percent body weight

rRSL did not differ from CONL for percent body weight either across the experiment or on individual days.

Discussion

To date, this is the first study to determine the effects of rRS on the reversal of spatial learning. Further, this is the first study to determine the effects of rRS administered during initial spatial learning on subsequent reversal learning in the Morris water maze. To address this, I used a comprehensive span of

measurements on both training and probe trials, including the Gallagher measures, previously shown to be more sensitive to group differences (Gallagher, Burwell et al. 1993; discussed in Hodges 1996).

In contrast to my hypotheses, I found that both immediate and delayed 6 hrs of RD during reversal learning did not significantly alter learning of the reversal platform location. Surprisingly, percent body weight was decreased for both rRSRev6-12 and CONR in comparison to rRSRev0-6 on Day 5, indicating that the immediately RD rats following reversal training were less stressed than either of the two control groups. I also did not find any altered performance in initial learning when rats were RS restricted during that initial spatial Learning Phase (Experiment 2). However, rRS during initial spatial learning did result in performance deficits on subsequent reversal learning (first 5 s cumulative distance and average proximity measures). Specifically, at the start of Day 5, rRSL had greater cumulative distance from the Reversal Phase platform during the first 5 s of the trialset, as compared to CONL. CONL also swam closer (average proximity error) to the Reversal Phase platform on the Day 6 probe, and overall had more platform crossings on both prior platform locations during the first 10 s of the probe trial. At the start of the Reversal Phase, on Day 4, rRSL swam faster than CONL, suggesting increased urgency or stress.

RS restriction during the Reversal Phase and initial Learning Phase

Interestingly, both reversal learning and initial spatial learning were similarly unaffected by RD immediately following 12 training trials per day. My results suggest that in the Morris water maze, rRS has no effect on the concurrent Learning Phase. These findings are in stark contrast to previous findings in the Morris water maze (Smith and Rose 1996; Smith and Rose 1997; Li, Tian et al. 2009; Wang, Huang et al. 2009), which describe a performance deficit following RD or rRS. Both Li et al. (2009) and Wang et al. (2009) used extensive periods of RD (24 hrs per day for 3 days), although RD was not begun until 3 days into the experiment. Unlike my 12 trials per day study, only 4 trials per day were administered in the Li and Wang studies, but their 3 day delay allowed a total of 12 trials prior to RD. As part of these two studies, performance was measured after recovery from the RD period, though surprisingly with differing results. Smith and Rose (1996; 1997) only used 4 hrs (instead of 6 hrs) of RD following training on either 4 trials per day (1996) or 12 trials (1997). For both Smith and Rose (1996; 1997) studies, latency was the only variable measured. The deficit in latency was only identified after the first period of rRS, but not on any other day of training and rRS (Smith and Rose 1996). This suggests that the rRS associated deficit is brief rather than occurring with each rRS session.

For those studies that found a deficit during spatial learning following either RD or rRS (Smith and Rose 1996; Smith and Rose, 1997; Li et al., 2009; Wang et al., 2009) the technique used to deprive the animals of RS was similar, but not

identical to mine. In the studies of Smith and Rose (1996; 1997); Smith et al. (1998); Bjorness et al. (2005); Ravassard et al. (2009); Li et al. (2009) and Wang et al. (2009) the inverted flowerpot technique (Jouvet, Vimont et al. 1964) was used where a rat would sit on an inverted flowerpot inside a tank with water in it. As the rat entered RS and had onset of muscle atonia they would begin to fall from the top of the inverted flowerpot and wake themselves up. Some of the studies (Smith and Rose 1996; Smith and Rose 1997; Smith, Conway et al. 1998; Li, Tian et al. 2009; Wang, Huang et al. 2009) used the inverted flowerpot technique with one inverted flowerpot in the chamber and a high level of water (up to 1 cm from the base of the platform), while others (Bjorness, Riley et al. 2005; Ravassard, Pachoud et al. 2009), including mine, used 3 inverted flowerpots and a low level of water (2 cm in the base of the tank). It is possible that my results differ from previous research in the Morris water maze as a result of the deprivation protocol, however, both Bjorness et al. (2005) and Ravassard et al. (2009) did find impairments in performance and learning. However, based on Ravassard et al.'s (2009) results, the RD chamber design I used in my current study may lead to only short impairments of LTP. In contrast, previous results using a shorter RD period, but a deprivation chamber more similar to that used in the previous studies with Morris water maze learning, found that LTP was impaired up to 24 hrs post-RD (Kim, Mahmoud et al. 2005). This suggests that the effects of RD through the deprivation method others have used (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997) could result in

prolonged negative effects on learning in contrast to the deprivation methods I used.

It has previously been shown that multiple inverted flowerpots are less stressful than a single inverted flowerpot (van Hulzen and Coenen 1981) as the rats can then move around and are not restricted. In my study, as compared to other RD studies using the Morris water maze, I used only three inverted flowerpots, which could suggest that my study was less stressful than those using only one (e.g. Smith and Rose 1996; Smith and Rose 1997). It is possible that the level of water could also result in differing levels of stress to the rats. Unlike RD with low level water, with high level water increased levels of muscle tone would be expected to maintain the head out of the water and the rat's tail would likely be in the water. Ravassard et al. (2009) also used 3 inverted flowerpots and a low level of water for their RD, and found no differences in stress levels with controls. Stress interferes with learning (Foy, Stanton et al. 1987; Bodnoff, Humphreys et al. 1995; Conrad, Galea et al. 1996; Krugers, Douma et al. 1997; McLay, Freeman et al. 1998). It is possible that the previously reported deficits in performance on spatial tasks may be the result of stress related to the RD technique rather than due to the RD itself. This suggests that findings from RD studies are fragile and should be carefully considered to determine the true effects of RD and potential contaminating side effects of the technique or general paradigm that may influence performance.

Another potential reason for my unexpected results is that 12 trials of learning within our Morris water maze may have resulted in 'overlearning' or a plateau effect. Although I used a similar learning load, of 12 trials, to Smith and Rose (1997) the richness of our room cues may have been more substantial, rendering this task solvable within the first 12 trials. My results indicated that learning improvements continued, indicating that a complete learning plateau had not been reached within the first 12 trials of this task. However, learning within the training period of the first day may have been sufficient to render the performance on the following day immune to any immediate effects of rRS. Aside from the potential effect of overlearning or complete learning, the Morris water maze can be solved without hippocampal dependent learning (Hoh, Beiko et al. 1999), suggesting the use of procedural strategies. Before RD was administered either after the first 12 trials on Day 1, for the initial Learning Phase, or after 42 Learning Phase and 6 Reversal Phase trials, for the Reversal Phase, rats may have had sufficient time to learn these procedural strategies and no longer rely on the hippocampus for spatial learning to solve this task. Further, procedural or habitual learning is thought to be unaffected by RD in rodents, and a change in reliance to a non-spatial strategy to solve a task has been previously seen following RD (Bjorness, Riley et al. 2005). It is possible that procedural learning or alternative strategy to solving the maze results in the lack of deficit observed for latency or pathlength in my study as compared to others (Smith and Rose 1997; Li, Tian et al. 2009; Wang, Huang et al. 2009) however, the cumulative distance measure is sensitive to search patterns which would reveal whether the

subjects were searching the target platform area as compared to using the procedural “sweep” method for solving the maze. I found that there were no differences, which would suggest that the rats were using similar (spatial) tactics. As there are no group differences during training in the cumulative distance from platform measure, I propose that none of the groups were purely reliant on non-spatial strategies.

Stress has long been measured during RD experiments in the form of percent body weight, levels of corticosterone, levels of ACTH and weight of the adrenal glands (e.g. van Hulzen and Coenen 1981; Suchecki, Lobo et al. 1998; Suchecki and Tufik 2000; Suchecki, Tiba et al. 2002; Machado, Hipolide et al. 2004). My study measured percent body weight as an indicator of stress. Although there were no group differences in behavior measured on Days 4 or 5, there was a significant change in body weight between these two days where CONR and rRSRev6-12 lost more body weight as compared to rRSRev0-6. This would suggest that rRSRev0-6 were less stressed than both of the other groups. It is understandable that delayed RD could be more stressful than an immediate RD period, as the rats are getting disturbed after they have settled down in their homecages (rRSRev6-12). However, I would have predicted that RD immediately following performance (rRSRev0-6) would be more stressful than being immediately returned to the homecages and remaining undisturbed until testing the next day (CONR). It is unclear why there was a loss in body weight for CONR as compared to rRSRev0-6. My results argue against a general stress effect of

rRS on learning in my study both because rats immediately RS restricted following learning did not display more stress indicators than controls, and because I did not find an initial learning deficit in RS restricted animals.

The effects of RS restriction on subsequent reversal learning

I found a rRS associated deficit in subsequent reversal learning during the probe trial (average proximity) and during the initial part of the training trials (first 5 s of cumulative distance). REM sleep restriction during learning may prevent the immediate consolidation of that learned platform, thus not allowing the hippocampus to be free of the first memory (Learning Phase platform location) before trying to establish the second (Reversal Phase platform location). Evidence for the time course of memory consolidation of a spatial task was shown by Kim and Fanselow (1992) that within 7 days, memories were significantly transferred outside the hippocampus elsewhere. Further, Poe et al. (2000) described a change in reactivation during REM sleep following spatial learning that suggested that consolidation and “clearing” of synapses for further spatial learning could occur three days into training. This was seen in a change in theta phase firing of hippocampal cells during RS depending on whether learning was familiar or novel. Further, they described that on the 4th day learning a previously novel task, the associated hippocampal cells no longer fired during the RS theta phase associated with novel tasks, but with the RS theta phase associated with familiar tasks. While the novel task’s associated hippocampal cell firing was at theta peaks, typically thought to be associated with LTP, familiar

associated hippocampal cell firing during RS was at theta troughs. Theta troughs are associated with depotentiation, or ‘unlearning’ within the hippocampus. Without RS, the change in theta phase dependent hippocampal cell firing may not occur. My results indicate that with rRS the second platform location or subsequent reversal platform location in this study cannot be learned on top of the first (Learning Phase platform location), perhaps because the first location has not been cleared from the synaptic network of the hippocampus. If this were the case, performance during the Learning Phase would not necessarily be impaired. However, with the introduction of a new platform location, the Learning Phase platform location could interfere with the new Reversal Phase platform location, resulting in a performance deficit, with both platform locations being represented at theta peaks as ‘novel’ platforms during RS. As mentioned earlier, Hasselmo et al. (2002) suggested that disruption to the theta rhythm could lead to the observed deficits in reversal learning.

I found that during the reversal probe, CONL swam in closer proximity to the reversal platform location as opposed to rRSL who tended to swim in closer proximity to the learning platform location. This suggests that rRS may result in less flexible learning, possibly a result of rRS altering the strength of proactive interference (Underwood 1957). In support of this recent evidence has indicated that RS facilitates flexible learning in humans (Wagner, Gais et al. 2004). Although further testing is required for the effect of rRS on flexible learning, it appears the initial learned platform location is more ‘hard-wired’ leaving the

animal less adaptable for learning new locations. The lack of flexibility in learning may be a result of the LTP / depotentiation balance being disrupted by previous RS deprivation, however this cannot be addressed in the current study. This result indicates while in many cases disruption to the hippocampus does not seem to lead to impairments in hippocampal-based learning, that this sometimes lack of measureable impairment may be limited to initial learning conditions only. It would be interesting to measure the effects of an additional platform location change in the rRSRev0-6 group as compared to CONR. Under the theoretical interpretation that rRS causes rigidity in learning, a deficit in learning a third platform location could be expected. However, a third experiment within the same environment (second reversal) could result in overlearning or a short-lived reliance on the hippocampus due to the existence of schemas based on prior learning experiences (Tse, Langston et al. 2007). Again, based on Hoh et al.'s work (1999), with such extensive prior learning within the environment, the rats would likely be able to solve the task in the absence of hippocampal based NMDA learning. Therefore, three learning experiences within the maze could render it impervious to hippocampal disruption.

At the time of reversal learning, groups, CONL and rRSL are in normal sleeping conditions. I do not expect the prior rRS for rRSL to result in any RS rebound due to the 3 days of 6 hrs of RD, as this amount of RD would not be expected to result in any lasting increases in RS pressure. I would expect the rRSL group to have recovered within the homecage period between RD and testing each day.

This expectation is supported by previous results indicating recovery from RD and resultant increases in compensatory RS within 4 hrs following 24 hr RD with low level water and 3 inverted flowerpots within the RD chamber (Mashour et al., *in review*). Thus I do not expect that rRS during learning would result in a prolonged RD recovery phase during reversal learning.

During the first 10 s of the probe trial, CONL had more platform crossings than rRSL. However, there was also a trend for CONL to have more learning platform crossings as compared to rRSL. These findings suggest that at the start of the probe on Day 6, CONL investigated both platform locations more than rRSL, which is indicative of better retention as shown by a difference in initial strategy and / or level of accuracy (number of platform crossings). With my current measurements, it is difficult to parse out the cause for these results. It is possible that rRSL either preferred the Learning Phase platform or were more disoriented when the platform was not in its expected Reversal Phase location. It is also possible, upon not finding the platform in the Reversal Phase location, CONL proceeded immediately to investigate the Learning Phase platform location. My results could also suggest that CONL had a higher level of accuracy, where they were able to swim through the exact locations of the previous two platform locations. In contrast, rRSL may have not learned either platform location to the same level of accuracy as CONL.

Some have intimated that the effect of sleep deprivation is similar to a temporary hippocampalectomy (Yoo, Hu et al. 2007). However, dentate gyrus granule cell loss was associated with a deficit in latency during initial spatial learning but not subsequent reversal learning in the Morris water maze (Conrad, Galea et al. 1996). Several potential reasons for the difference between my study and Conrad and Roy's findings exist. In my study, the effects of rRS are not necessarily restricted to the hippocampus and specifically not to the dentate gyrus. Further, rRS only acts as a temporary, reversible lesion of the hippocampus, which was only present for short periods of either experimental phase rather than throughout both phases of my study. If the damage to the dentate gyrus was sufficient to remove the ability to spatially learn the task, rats would have been forced to rely on other strategies independent of the hippocampus. These alternative strategies would have been well learned when reversal learning was introduced. Further, with a continued lesion in their hippocampus, their rats would have remained reliant on the learned compensatory strategies.

Results for reversal learning as compared to initial spatial learning appear very variable, with some studies describing reversal learning as faster to learn (Guzowski, Setlow et al. 2001), and others more difficult (Pouzet, Welzl et al. 1999). The rate of learning appears to be different between initial spatial learning and reversal learning, where reversal can be learned in 1 trial while naïve spatial learning may take 5 or 6 trials (Guzowski, Setlow et al. 2001). As the rats already

know the general strategies although not the platform location when starting reversal learning, there are fewer components to learn. That said, Pouzet et al. (1999) showed that performance during the first block of reversal learning was significantly poorer than the first block of spatial learning in the 8-arm maze. Therefore there does seem to be some discrepancy over the relative ease and general differences of reversal learning compared to initial spatial learning. My results may then contrast with those of Conrad and Roy because, if reversal learning is more difficult than initial learning, rRS effects may only be revealed with the more difficult task of reversal learning. However, this is an unlikely reason for my results, since I did not see the effect of rRS during concurrent reversal learning, only when an initial platform was learned under rRS.

General Discussion

A summary of my findings and potential theoretical explanations of my data are displayed in Figure 2.18. With 12 training trials per day, there was no change in performance with concurrent rRS, which may be the result of sufficient learning prior to the RS manipulation. Subsequent reversal learning, when the rats had undisturbed sleep following prior rRS during the previous initial spatial learning, showed performance deficits. These performance deficits for the previously rRS rats may be the result of a lack of depotentiation during the rRS periods leading to increased interference. Alternatively, previously rRS rats could have a decrease in flexibility in learning.

The Morris water maze may not be sensitive enough to be disrupted by initial RS manipulations. When the hippocampus was unilaterally inactivated, male rats had no deficits in performance for either Morris water maze learning or its reversal (Cimadevilla and Arias 2008). The group differences identified in my study are predominantly seen in the more sensitive Gallagher measures (Gallagher, Burwell et al. 1993; discussed in Hodges 1996; Maei, Zaslavsky et al. 2009) either for training trials (cumulative distance from the platform) or the probe trial (average proximity to the platform).

While it is possible that the mixed results previously found with the Morris water maze and hippocampal activity indicate that may not be a suitable testing tool for the effects of RD on hippocampus-dependent spatial learning and reversal learning, alternatively, it may be that experimenters need to expand their study to also include additional learning experiences. This is the first report on the effects of rRS on reversal learning in the Morris water maze. Additionally, this is the first report on the effects of rRS during initial spatial learning on subsequent reversal learning. My findings suggest that under this protocol, rRS does not hinder reversal learning or initial spatial learning. However, rRS during initial spatial learning does hinder subsequent reversal learning, perhaps indicating a deficit in flexibility of learning due to a disruption in the consolidation process and temporary network saturation. Future investigations are necessary to clarify the factors contributing to the contrasting results between my study and others that have described RD or rRS associated deficits during spatial learning.

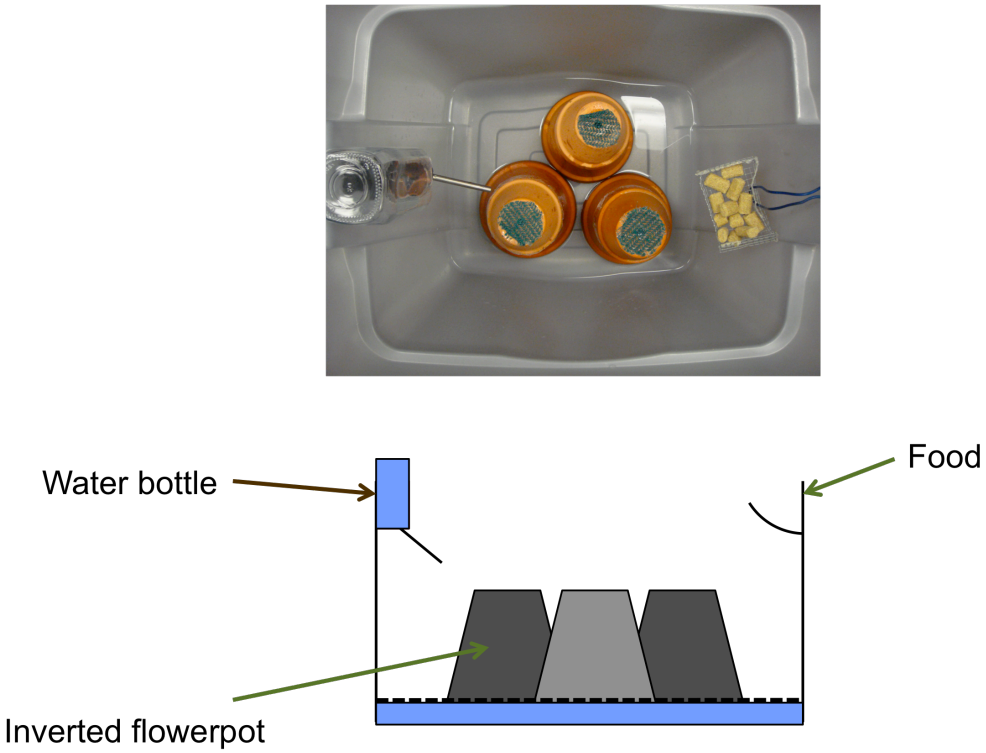
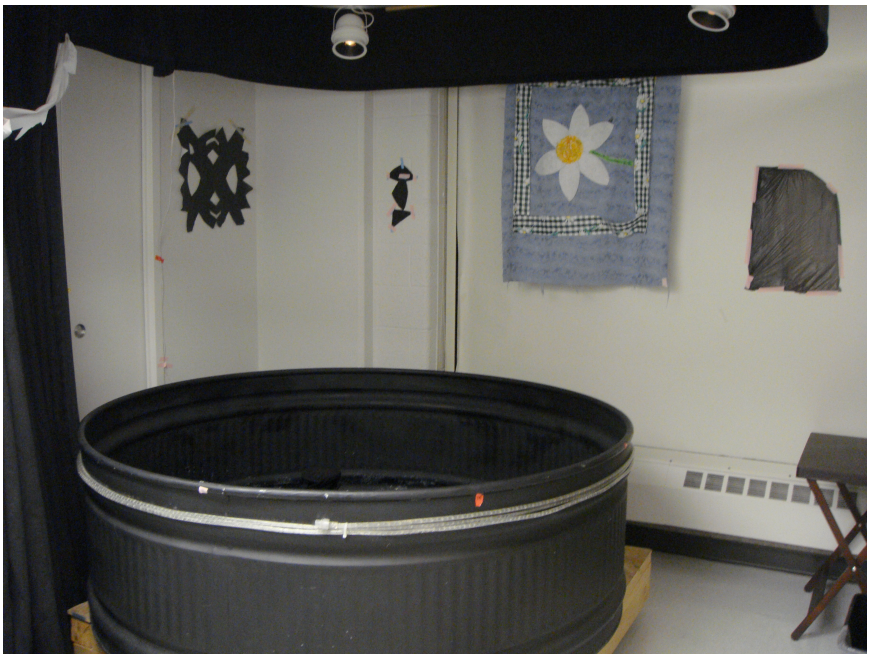


Figure 2.1 The inverted flower pot technique for REM sleep deprivation. The upper diagram is an overhead view of the deprivation chamber, while the lower diagram is a cross-sectional representation of the deprivation chamber. At the base of the chamber is 2 cm of water. In the center of the chamber are 3 inverted flowerpots. Water and food are freely available within the chamber.

A



B

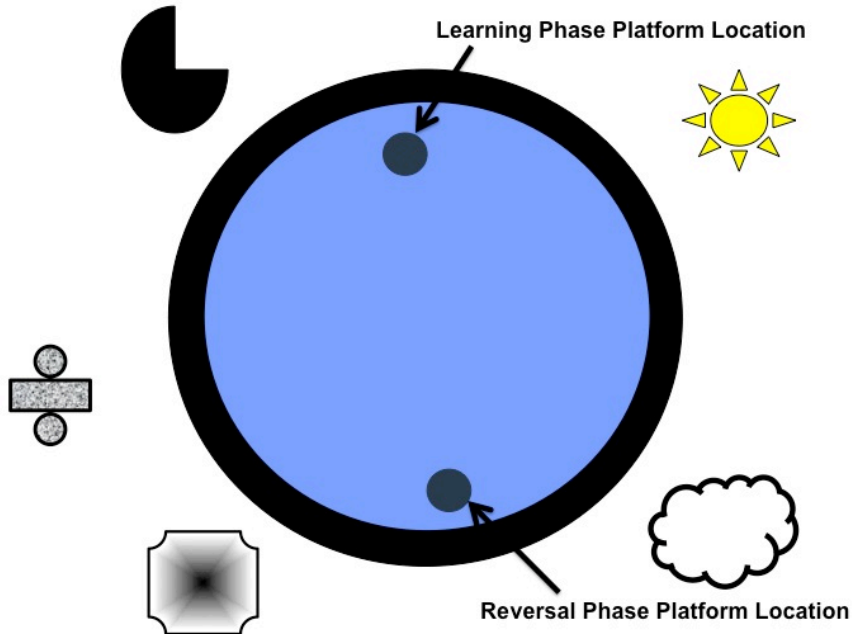


Figure 2.2 Morris water maze.
The Morris water maze is shown A) from a side view, and B) in an overhead cartoon format. Both platform locations can be seen in B, the initial platform location (Learning Phase platform location) and the second or reversed platform location (Reversal Phase platform location). Surrounding room cues can be seen in both A) and B).

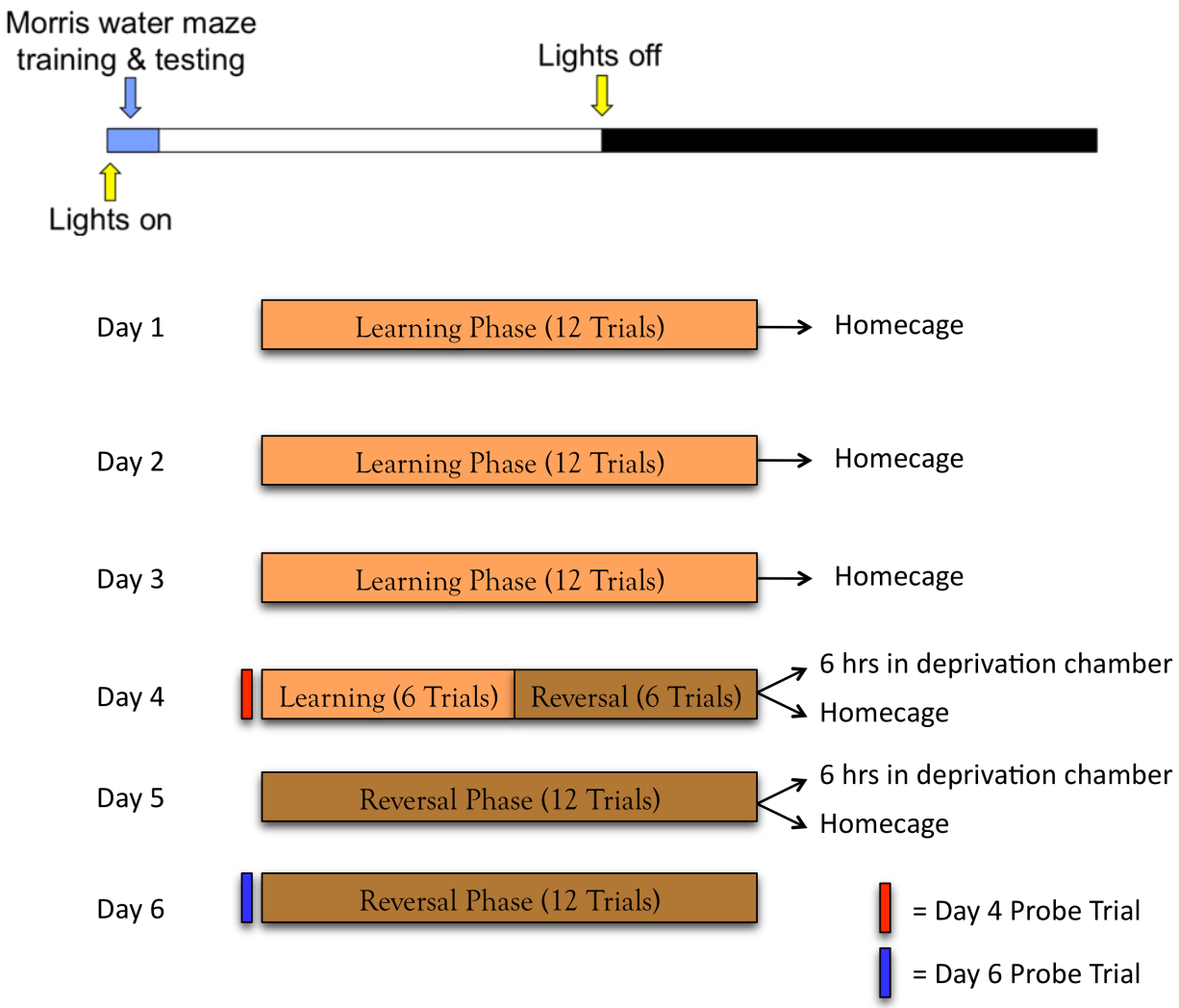


Figure 2.3 Experiment 1 protocol.
Across the 24 hr period, training or testing in the Morris water maze started shortly after lights on. There were 6 days within the protocol. Each day had 12 trials, with an additional probe trial on Days 4 and 6. At the start of Day 1, the rats were placed on the hidden platform for 20 s. The Reversal Phase started from the 7th trial Day 4 onwards. Probe trials are indicated as solid black rectangles. The initial habituation 20 s period is indicated as a solid grey rectangle. All rats on days 1, 2 and 3 were returned to their homecages, as were CONR on days 4 and 5. Following training on days 4 and 5, RD was administered. The rRSRev0-6 group underwent 6 hrs of REM deprivation immediately after the water maze. The rRSRev6-12 group underwent 6 hrs of REM deprivation starting 6 hrs after the water maze.

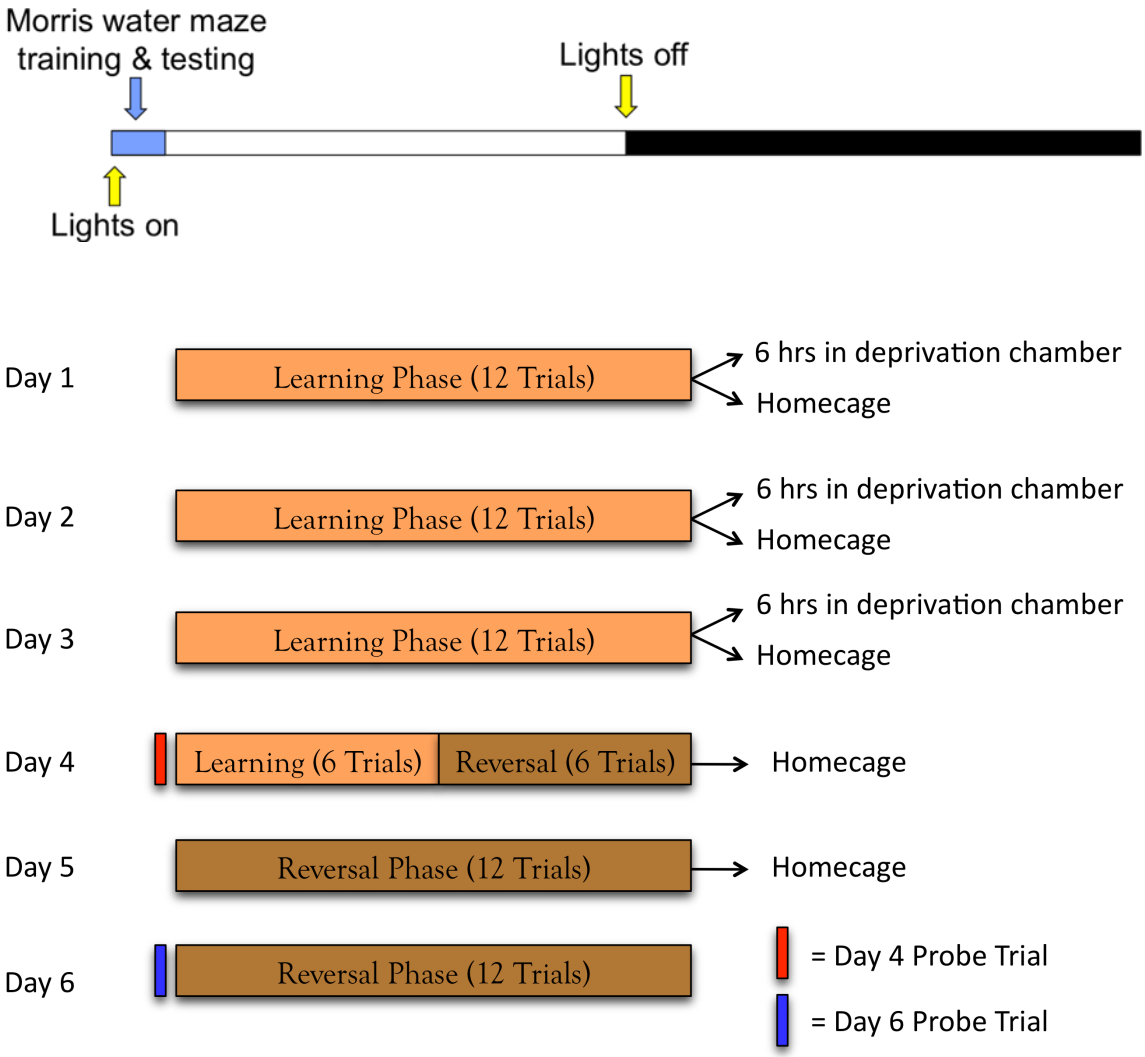


Figure 2.4 Experiment 2 protocol.
During the 24 hr period, training or testing in the Morris water maze was performed shortly after lights on. There were 6 days within the protocol. Each day had 12 trials, with an additional probe trial on Days 4 and 6. At the start of Day 1, the rats were placed on the hidden platform for 20 s. The Reversal Phase started from the 7th trial Day 4 onwards. Probe trials are indicated as solid black rectangles. The initial habituation 20 s period is indicated as a solid grey rectangle. rRSL were RD for 6 hrs immediately following training on Days 1, 2 and 3. On Days 4 and 5, all rats were returned to their homecages as were CONL on Days 1, 2 and 3.

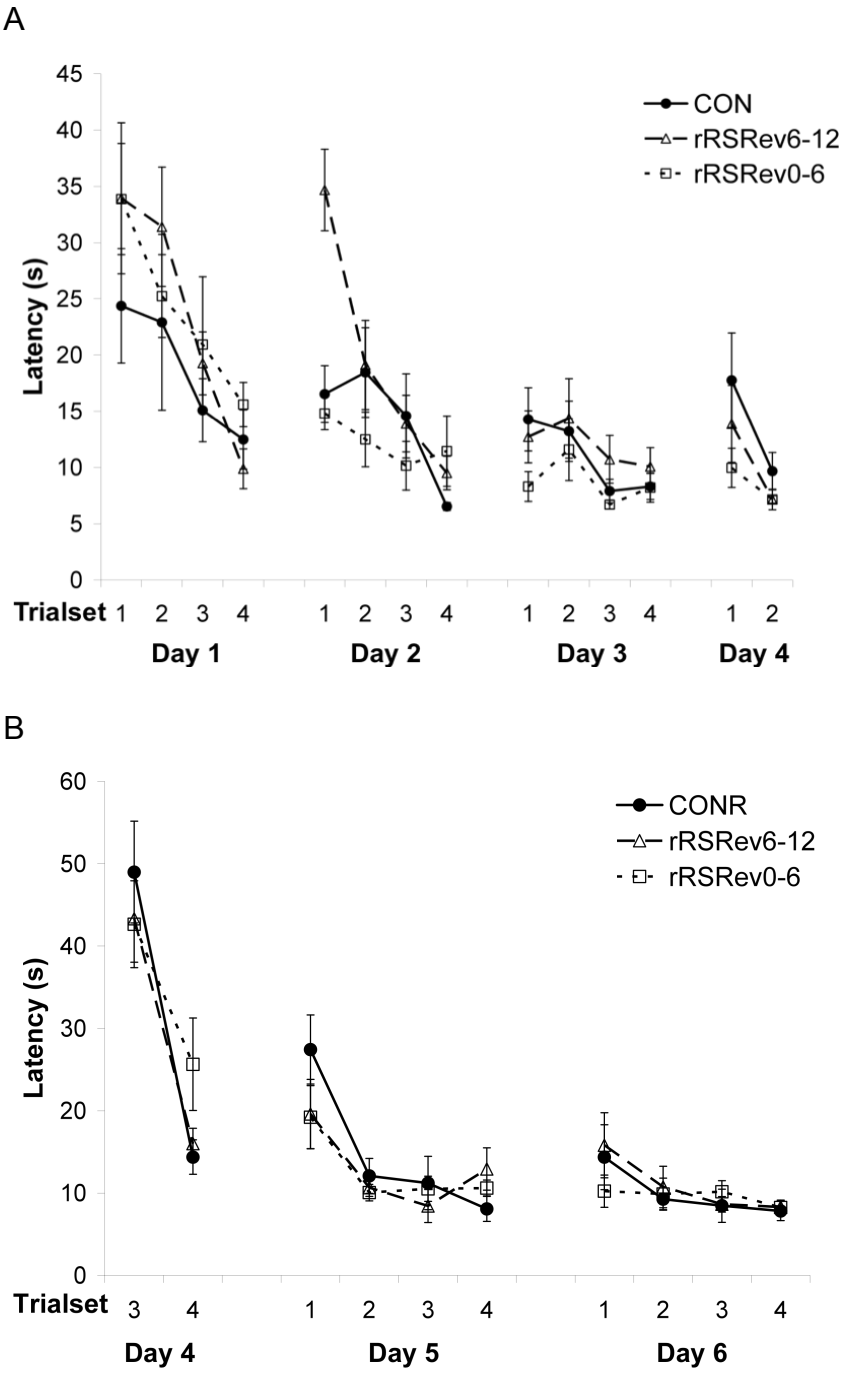


Figure 2.5 Experiment 1: Latency.
Reversal Phase data for Latency to platform are shown as mean ± SEM for CONR (solid black line), rRSRev6-12 (large dashed line) and rRSRev0-6 (small dashed line) across trialsets and days of A) the Learning Phase, and B) the Reversal Phase. rRS was during the Reversal Phase, following training on Days 4 and 5. No differences between groups were found for these measures. There was a trend for a group difference in retention for latency ($p = 0.057$), where CONR had poorer retention as compared to rRSRev0-6 ($p = 0.046$) was measured during the Reversal Phase.

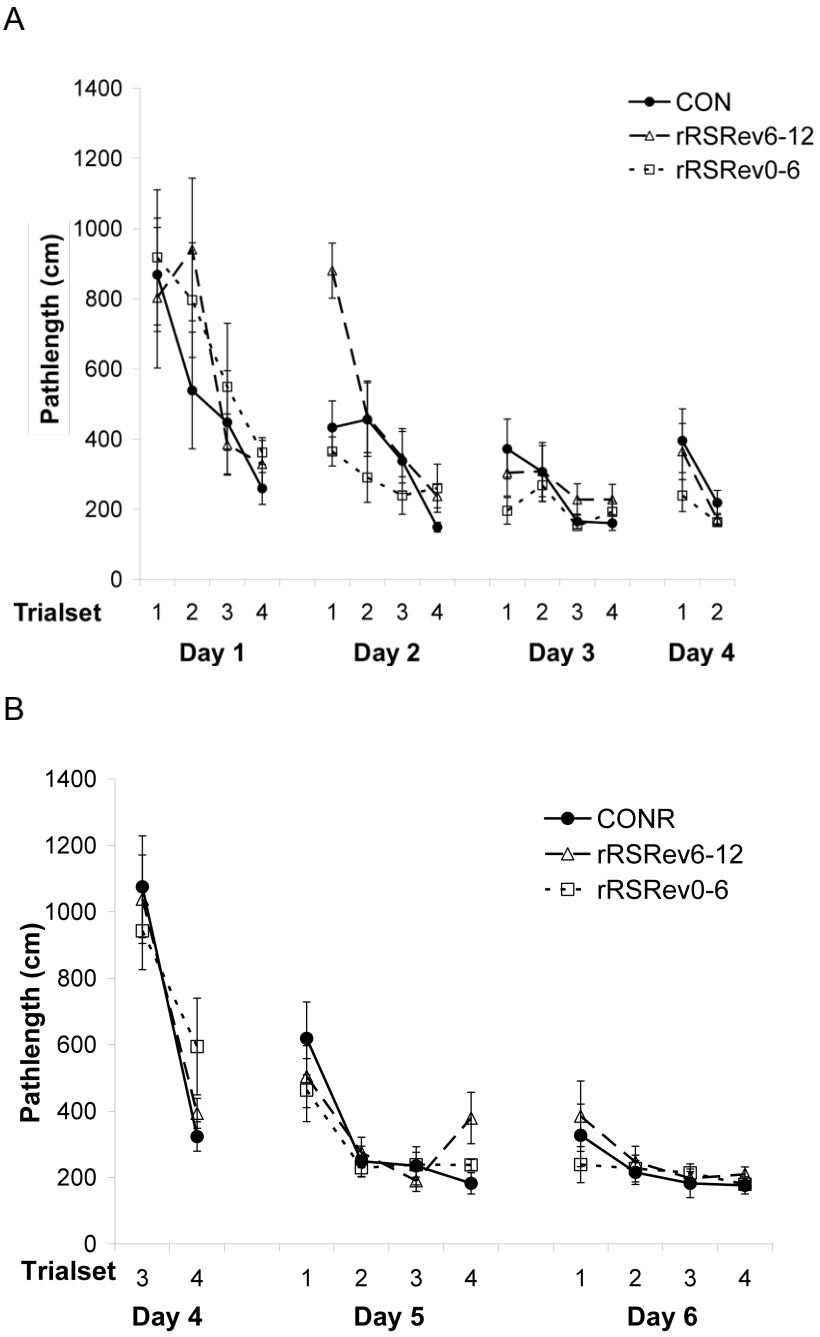
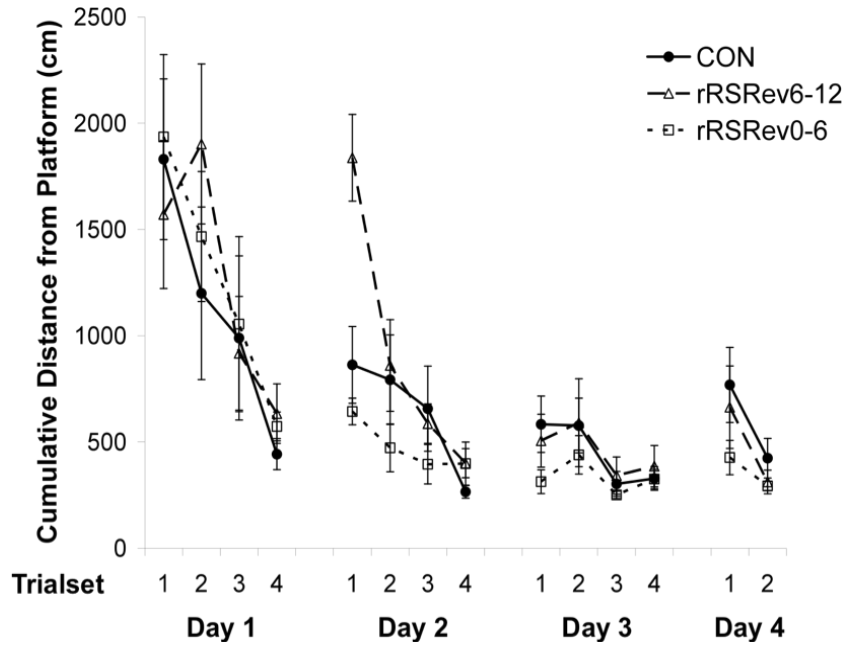


Figure 2.6 Experiment 1: Pathlength.
Pathlength data for A) the Learning Phase and B) the Reversal Phase are shown as mean ± SEM for CONR (solid black line), rRSRev6-12 (large dashed line) and rRSRev0-6 (small dashed line) across days and trialsets. rRS was during the Reversal Phase, following training on Days 4 and 5. No differences between groups were found for these measures. There was a trend for a group difference in retention for latency ($p = 0.057$), where CONR had poorer retention as compared to rRSRev0-6 ($p = 0.046$) during the Reversal Phase.

A



B

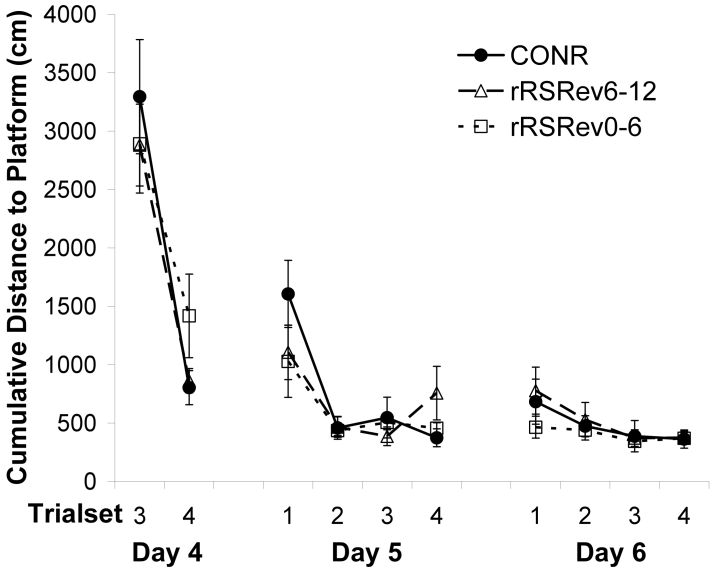


Figure 2.7 Experiment 1: Cumulative distance from the platform.
Cumulative distance from A) the Learning Phase platform location and B) the Reversal Phase platform location data are shown as mean ± SEM for CONR (solid black line), rRSRev6-12 (large dashed line) and rRSRev0-6 (small dashed line) across days and trialsets. rRS was during the Reversal Phase, following training on Days 4 and 5. No differences between groups were found for this measure.

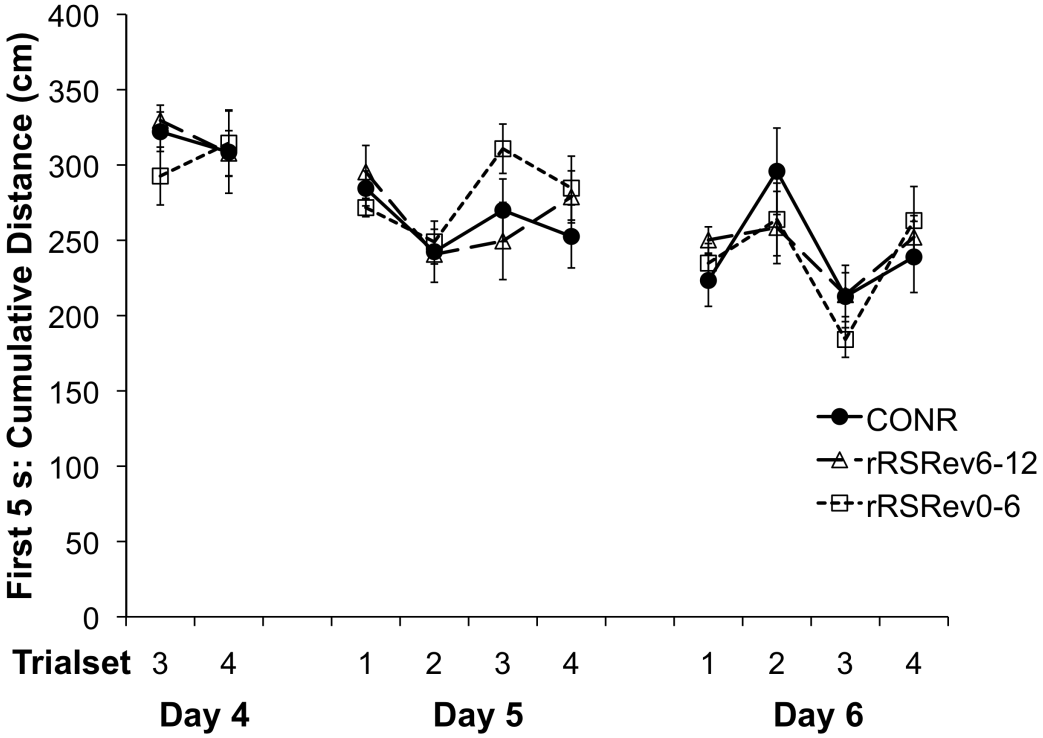


Figure 2.8 Experiment 1: First 5 s of trials for Cumulative distance from the platform across the Reversal Phase.

First 5 s of cumulative distance from the Reversal Phase platform location are shown as mean ± SEM for CONR (solid black line), rRSRev6-12 (large dashed line) and rRSRev0-6 (small dashed line) across the three reversal days: Day 4 (2 trialsets) and Days 5 and 6 (4 trialsets). rRS was during the Reversal Phase, following training on Days 4 and 5. No differences between groups were found for these measured.

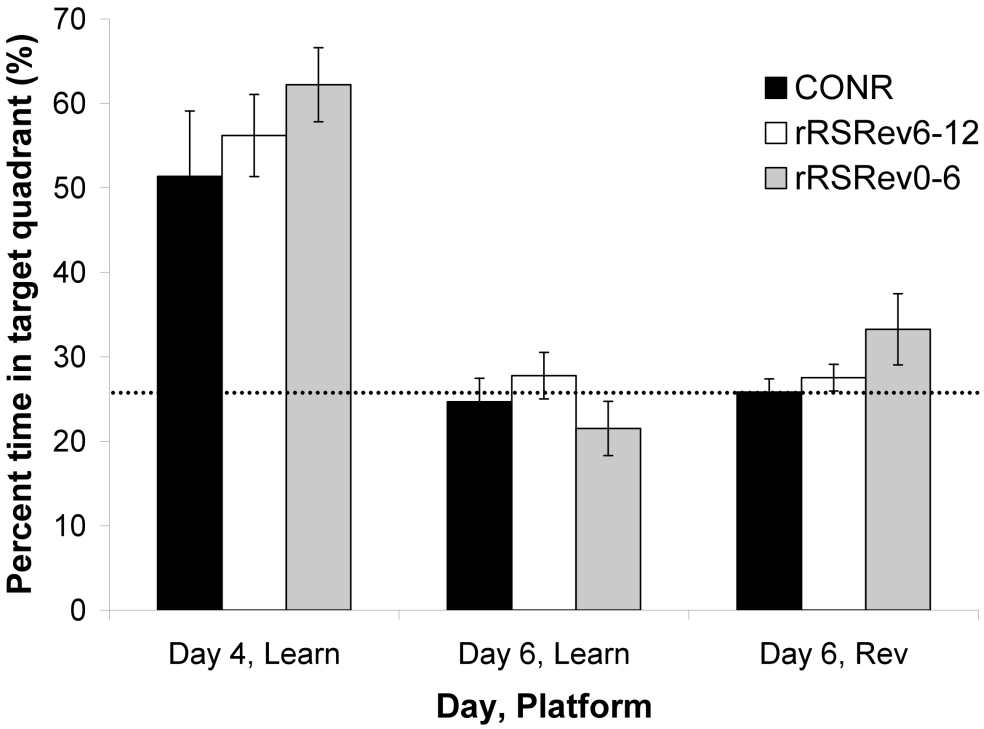


Figure 2.9 Experiment 1: Percent time spent in target quadrant during the probe trials.

Percent time spent in the target quadrant for the probe trials on Days 4 and 6 are shown as mean ± SEM for CONR (black), rRSRev6-12 (white) and rRSRev0-6 (grey) for the Learning Phase quadrant (Learn) and the Reversal Phase quadrant (Rev). The dashed line indicates chance (25 %). rRS was during the Reversal Phase, following training on Days 4 and 5.

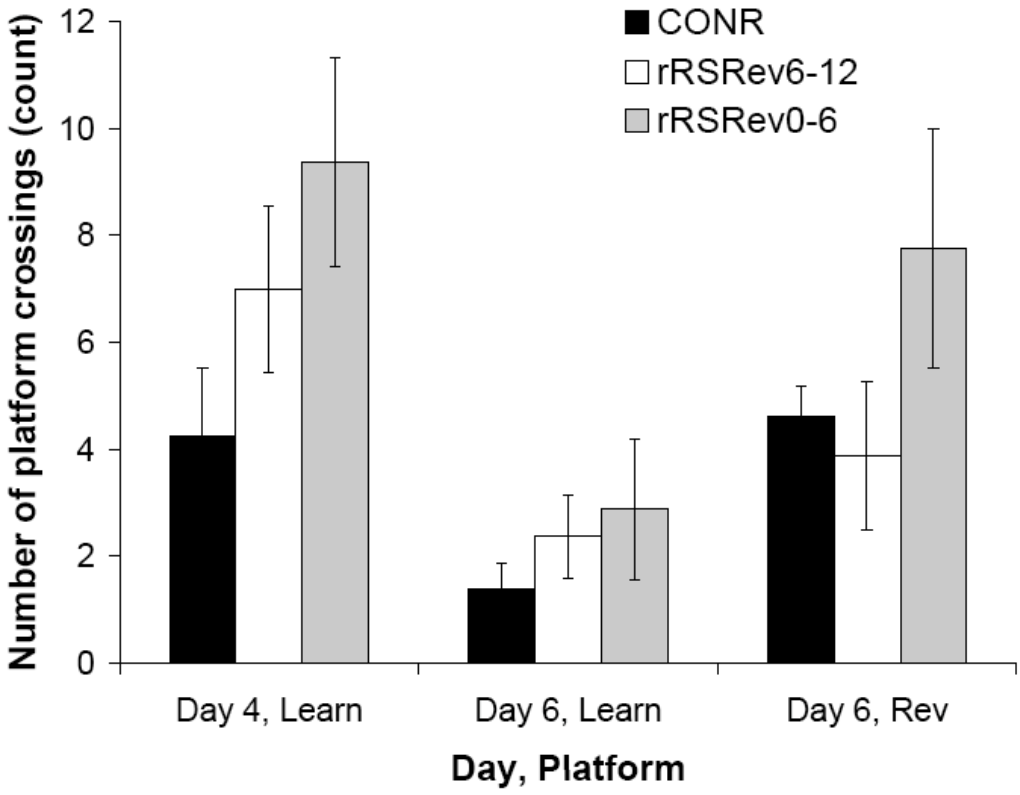


Figure 2.10 Experiment 1: Number of platform crossings during the probe trials.

Number of platform crossings for the probe trials on Days 4 and 6 are shown as mean ± SEM for CONR (black), rRSRev6-12 (white) and rRSRev0-6 (grey) for the Learning Phase platform location (Learn) and the Reversal Phase platform location (Rev). rRS was during the Reversal Phase, following training on Days 4 and 5.

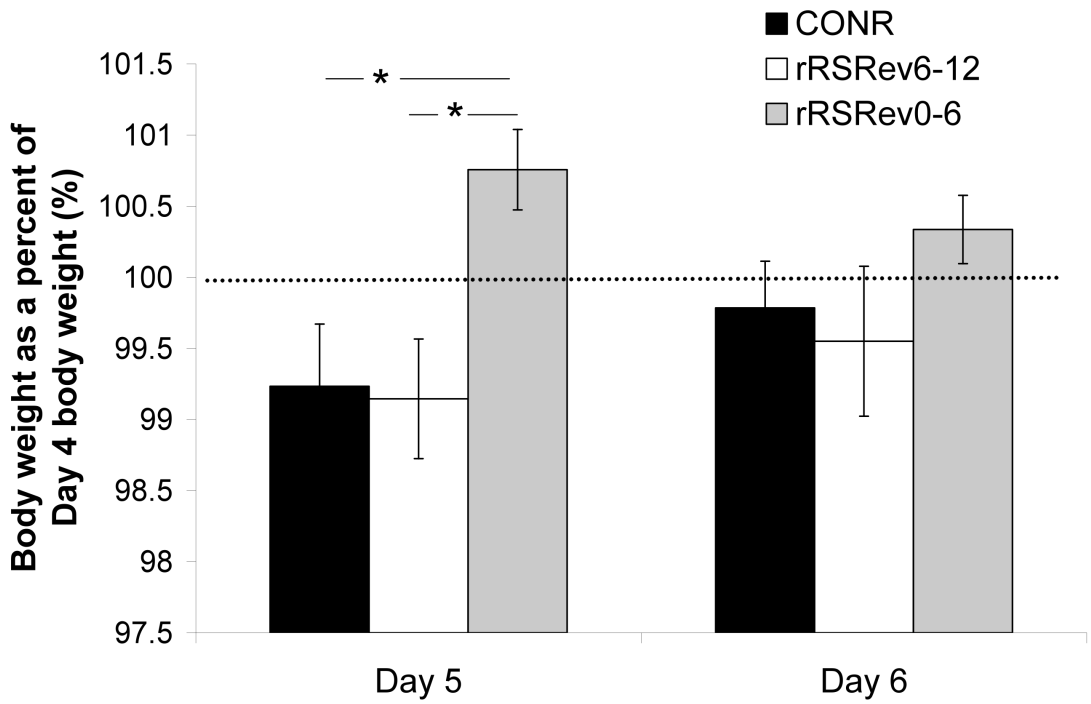


Figure 2.11 Experiment 1: Percent body weight
Body weight is shown for the entire experiment as a percentage of Day 4 body weight. Data are shown as mean ± SEM for CONR (black), rRSRev6-12 (white) and rRSRev0-6 (grey). rRS was during the Reversal Phase, following training on Days 4 and 5. A group main effect ($p = 0.012$) was measured where both CONR ($p = 0.029$) and rRSRev6-12 ($p = 0.02$) had lost more percent body weight than rRSRev0-6 on Day 5 as a percent of Day 4. * $p < 0.05$.

Table 2.1 Summary of the behavioral results for rRS concurrent with the Reversal Phase as compared to controls

	rRSRev6-12	rRSRev0-6
Training	Reversal Phase	Reversal Phase
Latency	-	* Recall: better than CONR
Pathlength	-	-
Cumulative Distance	-	# Recall: better than CONR
5s Cumulative Distance	-	-
Swim Speed	-	-
Probe Trial		
60 s Percent time in Learning Phase Quadrant	-	-
60 s Percent time in Reversal Phase Quadrant	-	-
60 s Number of Learning Phase Platform Crossings	-	-
60 s Number of Reversal Phase Platform Crossings	-	-
60 s Avg. Proximity to Learning Phase Platform	-	-
60 s Avg. Proximity to Reversal Phase Platform	-	-
10 s Probe Percent time in target quadrant	-	-
10 s Number of platform crossings	-	-
10 s Probe Avg Proximity to target platform	-	-
Swim Speed	-	-

Results are shown for comparisons to CONR. * $p < 0.05$. # $p < 0.1$

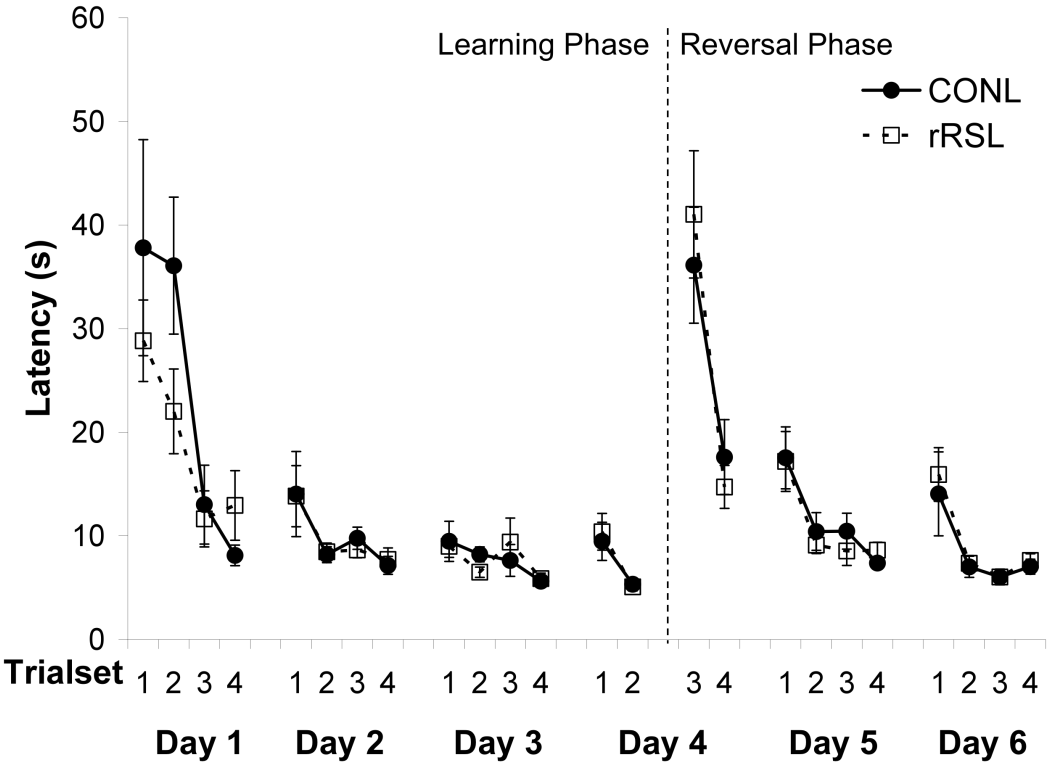


Figure 2.12 Experiment 2: Latency
Latency for CONL and rRSL for both the Learning Phase and subsequent Reversal Phase is shown as mean ± SEM for CONL (solid line, solid circle) and rRSL (dashed line, open square) across the Learning and Reversal Phase. rRS was during the Learning Phase, following training on Days 1, 2 and 3. No group differences were identified.

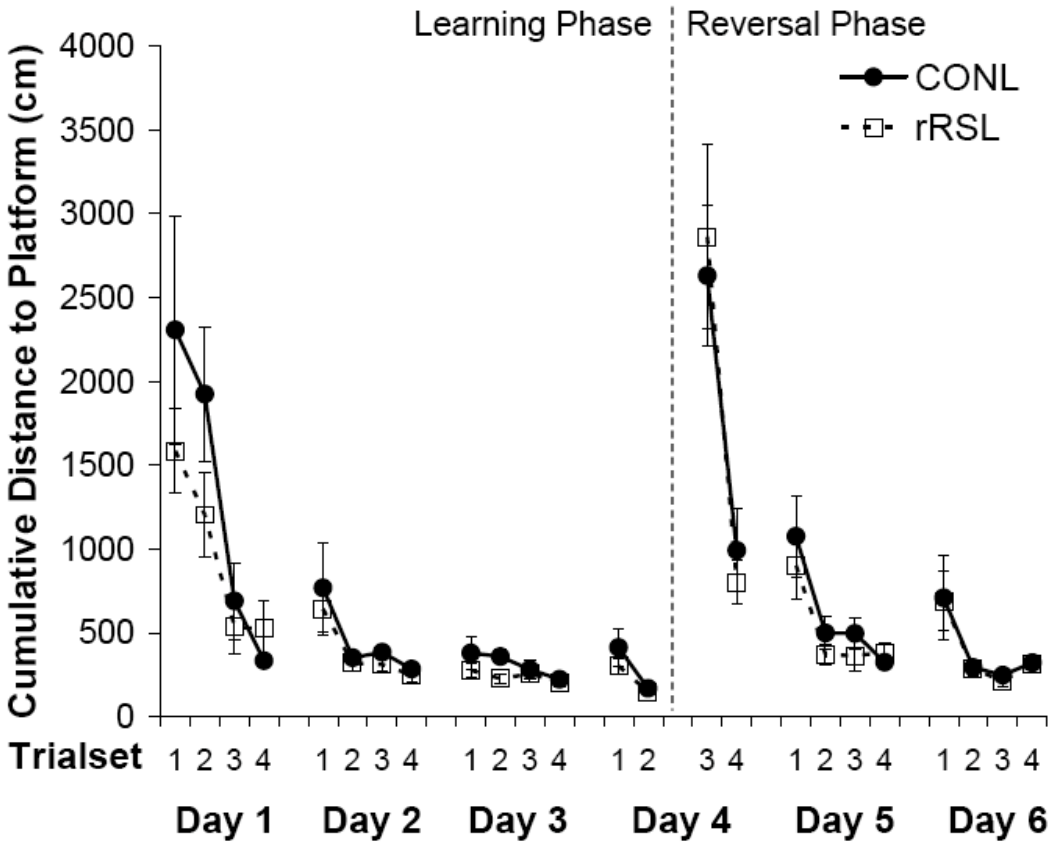


Figure 2.13 Experiment 2: Cumulative distance from target platform
Cumulative distance from the Learning Phase (Days 1, 2, 3 and 4) or the Reversal Phase (Days 4, 5 and 6) platform location for CONL and rRSL is shown as mean ± SEM for CONL (solid line, solid circle) and rRSL (dashed line, open square). rRS was during the Learning Phase, following training on Days 1, 2 and 3. No group differences were identified.

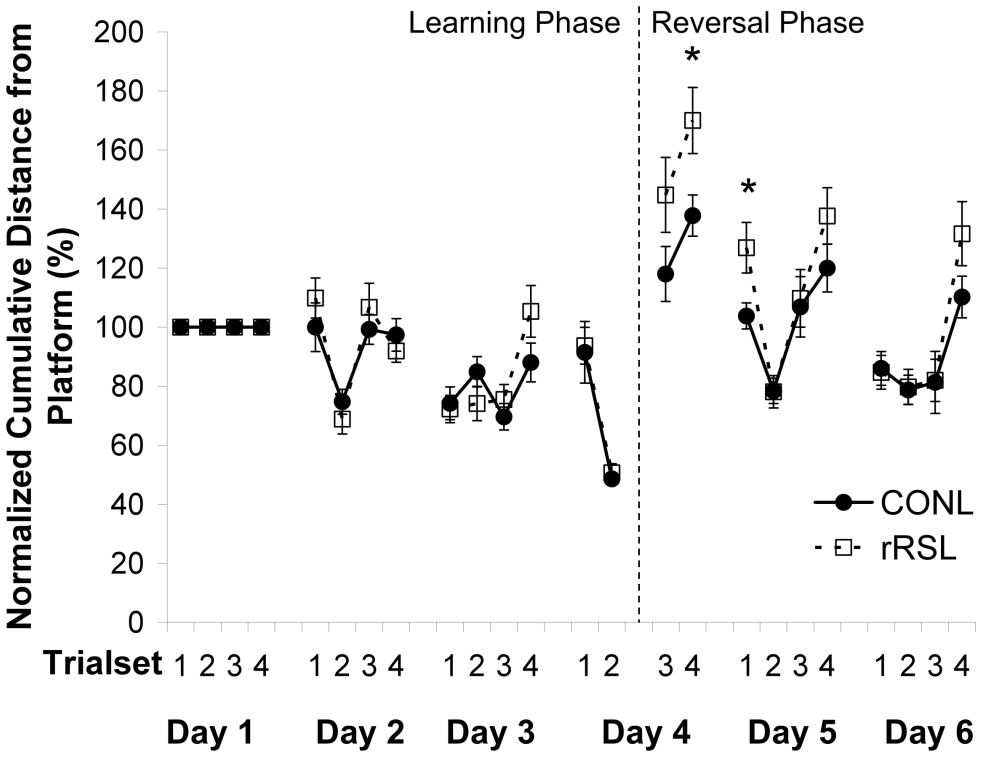


Figure 2.14 Experiment 2: First 5 s of Cumulative distance from target platform

The first 5 s of cumulative distance from the Learning Phase (Days 1, 2, 3 and 4) or the Reversal Phase (Days 4, 5 and 6) platform location for CONL and rRSL is shown as mean ± SEM for CONL (solid line, solid circle) and rRSL (dashed line, open square). rRS was during the Learning Phase, following training on Days 1, 2 and 3. rRSL performed significantly poorer than CONL at the end of Day 4 and the start of Day 5, during subsequent reversal learning. * $p < 0.05$.

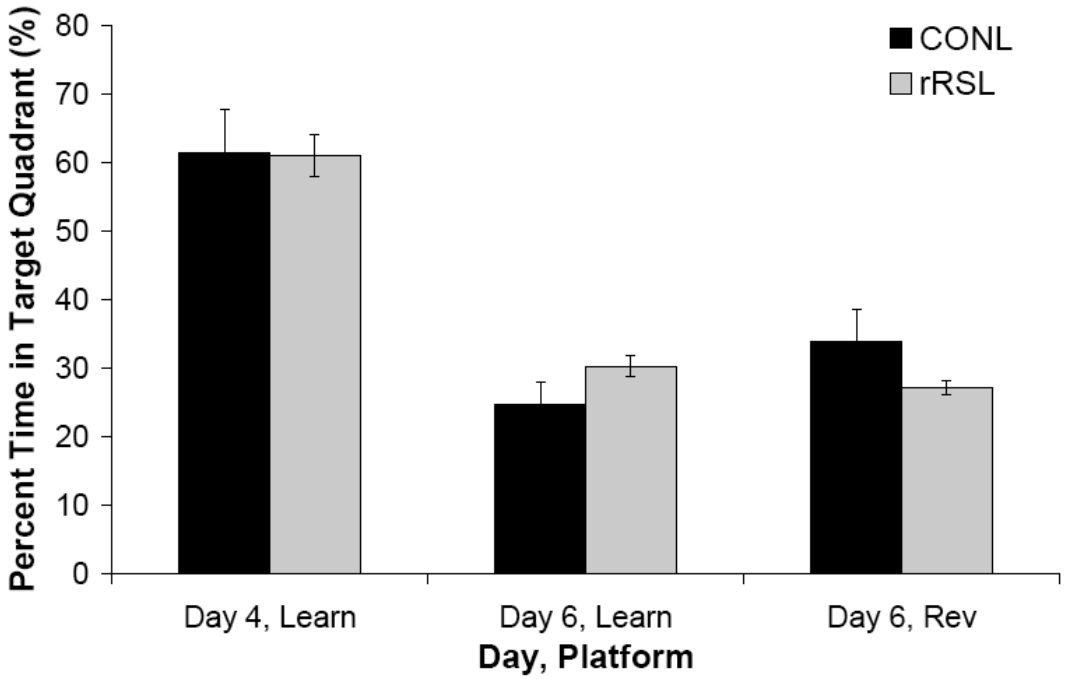


Figure 2.15 Experiment 2 Percent time spent in target quadrant during the probe trials

Percent time spent in either the Learning Phase (Learn) or the Reversal Phase (Rev) quadrants on the Day 4 and Day 6 probe trial is shown as mean ± SEM for CONL (black) and rRSL (grey). rRS was during the Learning Phase, following training on Days 1, 2 and 3.

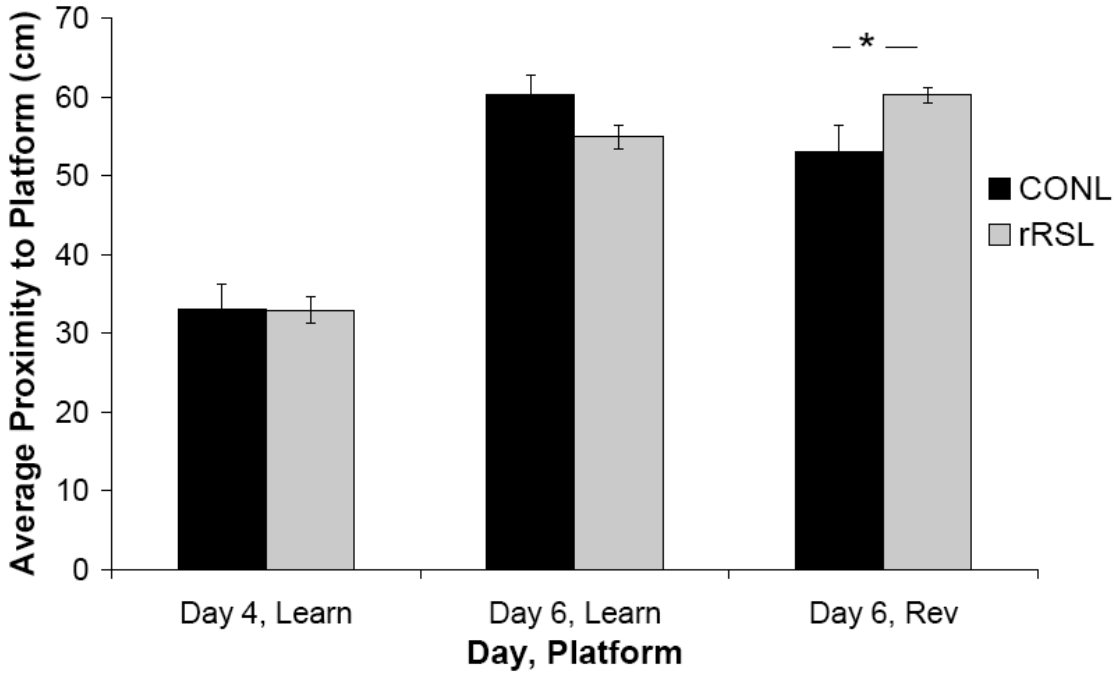


Figure 2.16 Average proximity to target platform during the probe trial
Average proximity to the Learning Phase (Learn) and Reversal Phase (Rev) platform location is shown as mean ± SEM for CONL (black) and rRSL (grey) on Days 4 and 6. rRS was during the Learning Phase, following training on Days 1, 2 and 3. * $p < 0.05$.

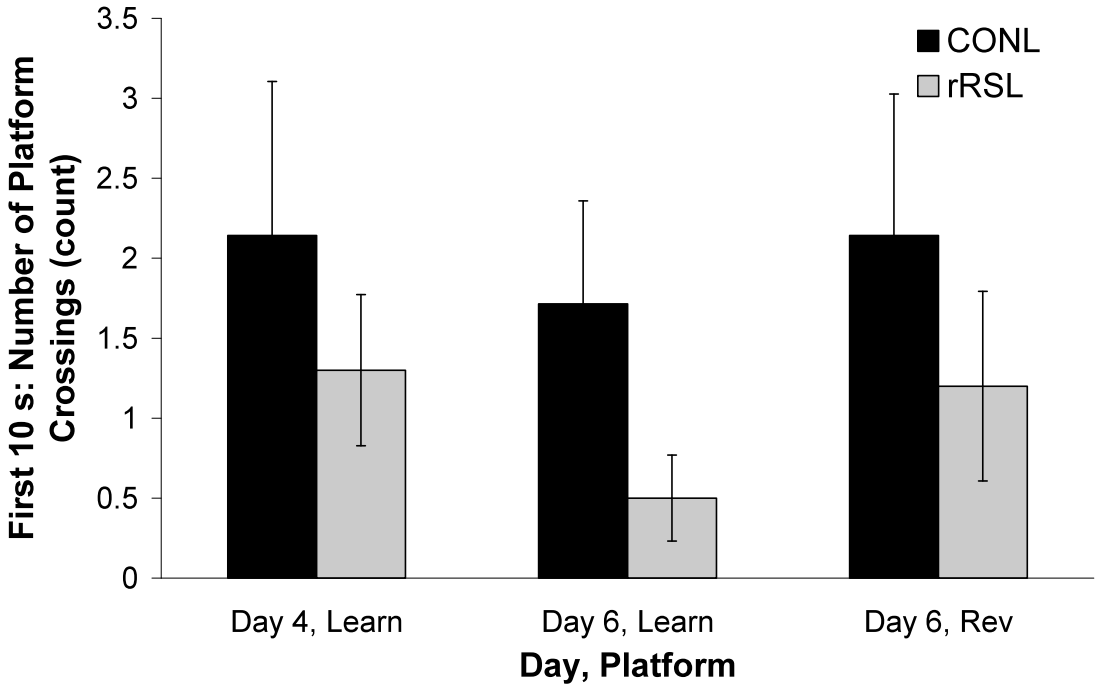


Figure 2.17 Number of platform crossings during the probe trial
The first 10 s of the number of platform crossings for the Learning Phase (Learn) and Reversal Phase (Rev) platform locations are shown as mean ± SEM for CONL (black) and rRSL (grey) on both the Day 4 and Day 6 probe trial. rRS was during the Learning Phase, following training on Days 1, 2 and 3.

Table 2.2 A Summary of the effects of rRS during the Learning Phase

	rRSL
Training	Learning Phase
Latency	-
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	-
Swim Speed	-
Probe Trial	
60 s Percent time in Learning Phase Quadrant	-
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	-
60 s Avg. Proximity to Reversal Phase Platform	-
10 s Probe Percent time in target quadrant	-
10 s Number of platform crossings	-
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

Table 2.2 B Summary of the effects of prior rRS on Reversal Phase performance

	rRSL
Training	Reversal Phase
Latency	-
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	* Worse than CONL
Swim Speed	* Faster than CONL
Probe Trial	
60 s Percent time in Learning Phase Quadrant	-
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	# Swam closer than CONL
60 s Avg. Proximity to Reversal Phase Platform	* Swam further away than CONL
10 s Probe Percent time in target quadrant	# (L.P. & R.P.) Less time than CONL
10 s Number of platform crossings	* (L.P. & R.P.) Fewer crossings than CONL
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

L.P. Learning Phase, R.P. Reversal Phase. * $p < 0.05$. # $p < 0.1$

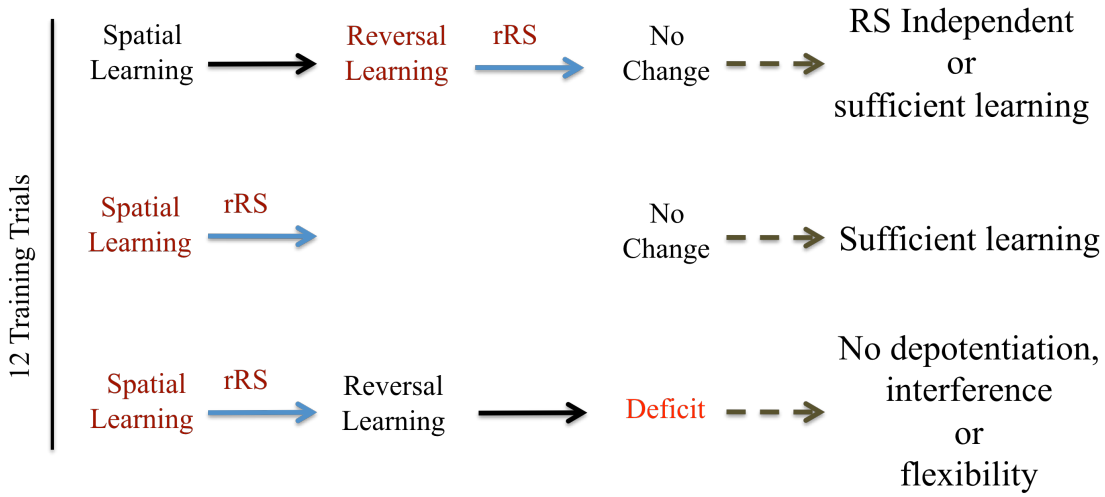


Figure 2.18 Summary of the results fro Experiments 1 and 2.
Results are shown for 12 training trials per day, for Experiment 1 with rRS during reversal learning (Reversal Phase) and for Experiment 2 with rRS during the initial spatial learning (Learning Phase). In experiments 1 and 2 there was no change in performance with concurrent learning. This may have been the result of sufficient learning prior to the RS manipulation. In Experiment 2, there was an observed lack of preference for the reversed platform location in previously rRS rats. This may be the result of increased interference due to a lack of depotentiation during the rRS period or decreased flexibility in learning for previously rRS rats. Phase during rRS (burgundy), performance enhancements (green), performance deficits (red).

References

Bjorness, T. E., Riley, B. T., Tysor, M. K., & Poe, G. R. (2005). REM restriction persistently alters strategy used to solve a spatial task. *Learn Mem, 12*(3), 352-359.

Blokland, A., de Vente, J., Prickaerts, J., Honig, W., Markerink-van Ittersum, M., & Steinbusch, H. (1999). Local inhibition of hippocampal nitric oxide synthase does not impair place learning in the Morris water escape task in rats. *Eur J Neurosci, 11*(1), 223-232.

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., & Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J Neurosci, 15*(1 Pt 1), 61-69.

Bramham, C. R., Maho, C., & Laroche, S. (1994). Suppression of long-term potentiation induction during alert wakefulness but not during 'enhanced' REM sleep after avoidance learning. *Neuroscience, 59*(3), 501-509.

Cimadevilla, J. M., & Arias, J. L. (2008). Different vulnerability in female's spatial behaviour after unilateral hippocampal inactivation. *Neurosci Lett, 439*(1), 89-93.

Cirulli, F., Berry, A., & Alleva, E. (2000). Intracerebroventricular administration of brain-derived neurotrophic factor in adult rats affects analgesia and spontaneous behaviour but not memory recall in a Morris Water Maze task. *Neurosci Lett, 287*(3), 207-210.

Cirulli, F., Berry, A., Chiarotti, F., & Alleva, E. (2004). Intrahippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze. *Hippocampus, 14*(7), 802-807.

Conrad, C. D., Galea, L. A., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. *Behav Neurosci, 110*(6), 1321-1334.

Conrad, C. D., & Roy, E. J. (1993). Selective loss of hippocampal granule cells following adrenalectomy: implications for spatial memory. *J Neurosci, 13*(6), 2582-2590.

Davis, C. J., Harding, J. W., & Wright, J. W. (2003). REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus. *Brain Res, 973*(2), 293-297.

Fishbein, W., Kastaniotis, C., & Chattman, D. (1974). Paradoxical sleep: prolonged augmentation following learning. *Brain Res, 79*(1), 61-75.

Foy, M. R., Stanton, M. E., Levine, S., & Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behav Neural Biol, 48*(1), 138-149.

Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., et al. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in

hippocampus-independent tasks in rats. *Neuroscience, 144*(4), 1186-1192.
Gallagher, M., Burwell, R., & Burchinal, M. (1993). Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci, 107*(4), 618-626.
Guzowski, J. F., Setlow, B., Wagner, E. K., & McGaugh, J. L. (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. *J Neurosci, 21*(14), 5089-5098.
Harrell, L. E., Barlow, T. S., Miller, M., Haring, J. H., & Davis, J. N. (1984). Facilitated reversal learning of a spatial-memory task by medial septal injections of 6-hydroxydopamine. *Exp Neurol, 85*(1), 69-77.
Hasselmo, M. E., Bodelon, C., & Wyble, B. P. (2002). A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. *Neural Comput, 14*(4), 793-817.
Hicks, R. A., Okuda, A., & Thomsen, D. (1977). Depriving rats of REM sleep: the identification of a methodological problem. *Am J Psychol, 90*(1), 95-102.
Hodges, H. (1996). Maze procedures: the radial-arm and water maze compared. *Brain Res Cogn Brain Res, 3*(3-4), 167-181.
Hoh, T., Beiko, J., Boon, F., Weiss, S., & Cain, D. P. (1999). Complex behavioral strategy and reversal learning in the water maze without NMDA receptor-dependent long-term potentiation. *J Neurosci, 19*(10), RC2.
Ishikawa, A., Kanayama, Y., Matsumura, H., Tsuchimochi, H., Ishida, Y., & Nakamura, S. (2006). Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus. *Eur J Neurosci, 24*(1), 243-248.
Jouvet, D., Vimont, P., & Delorme, F. (1964). [Study of Selective Deprivation of the Paradoxal Phase of Sleep in the Cat.]. *J Physiol (Paris), 56*, 381.
Joyal, C. C., Strazielle, C., & Lalonde, R. (2001). Effects of dentate nucleus lesions on spatial and postural sensorimotor learning in rats. *Behav Brain Res, 122*(2), 131-137.
Kim, E. Y., Mahmoud, G. S., & Grover, L. M. (2005). REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus. *Neurosci Lett, 388*(3), 163-167.
Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*(5057), 675-677.
Krugers, H. J., Douma, B. R., Andringa, G., Bohus, B., Korf, J., & Luiten, P. G. (1997). Exposure to chronic psychosocial stress and corticosterone in the rat: effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity. *Hippocampus, 7*(4), 427-436.
Lacroix, L., White, I., & Feldon, J. (2002). Effect of excitotoxic lesions of rat medial prefrontal cortex on spatial memory. *Behav Brain Res, 133*(1), 69-81.
Leconte, P., Hennevin, E., & Bloch, V. (1974). Duration of paradoxical sleep necessary for the acquisition of conditioned avoidance in the rat. *Physiol Behav, 13*(5), 675-681.

Li, S., Tian, Y., Ding, Y., Jin, X., Yan, C., & Shen, X. (2009). The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats. *Learn Behav, 37*(3), 246-253.

Machado, R. B., Hipolide, D. C., Benedito-Silva, A. A., & Tufik, S. (2004). Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. *Brain Res, 1004*(1-2), 45-51.

Maei, H. R., Zaslavsky, K., Teixeira, C. M., & Frankland, P. W. (2009). What is the Most Sensitive Measure of Water Maze Probe Test Performance? *Front Integr Neurosci, 3*, 4.

Mavanji, V., & Datta, S. (2003). Activation of the phasic pontine-wave generator enhances improvement of learning performance: a mechanism for sleep-dependent plasticity. *Eur J Neurosci, 17*(2), 359-370.

McDermott, C. M., Hardy, M. N., Bazan, N. G., & Magee, J. C. (2006). Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus. *J Physiol, 570*(Pt 3), 553-565.

McGrath, M. J., & Cohen, D. B. (1978). REM sleep facilitation of adaptive waking behavior: a review of the literature. *Psychol Bull, 85*(1), 24-57.

McLay, R. N., Freeman, S. M., & Zadina, J. E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. *Physiol Behav, 63*(5), 933-937.

Morris, R. G., Hagan, J. J., & Rawlins, J. N. (1986). Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function. *Q J Exp Psychol B, 38*(4), 365-395.

Pearlman, C. (1973). REM sleep deprivation impairs latent extinction in rats. *Physiol Behav, 11*(2), 233-237.

Poe, G. R., Nitz, D. A., McNaughton, B. L., & Barnes, C. A. (2000). Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. *Brain Res, 855*(1), 176-180.

Portell-Cortes, I., Marti-Nicolovius, M., Segura-Torres, P., & Morgado-Bernal, I. (1989). Correlations between paradoxical sleep and shuttle-box conditioning in rats. *Behav Neurosci, 103*(5), 984-990.

Pouzet, B., Welzl, H., Gubler, M. K., Broersen, L., Veenman, C. L., Feldon, J., et al. (1999). The effects of NMDA-induced retrohippocampal lesions on performance of four spatial memory tasks known to be sensitive to hippocampal damage in the rat. *Eur J Neurosci, 11*(1), 123-140.

Ravassard, P., Pachoud, B., Comte, J. C., Mejia-Perez, C., Scote-Blachon, C., Gay, N., et al. (2009). Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. *Sleep, 32*(2), 227-240.

Romcy-Pereira, R., & Pavlides, C. (2004). Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP. *Eur J Neurosci, 20*(12), 3453-3462.

Ruskin, D. N., Dunn, K. E., Billiot, I., Bazan, N. G., & LaHoste, G. J. (2006). Eliminating the adrenal stress response does not affect sleep deprivation-induced acquisition deficits in the water maze. *Life Sci, 78*(24), 2833-2838.
Silvestri, A. J. (2005). REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiol Behav, 84*(3), 343-349.
Smith, C., & Butler, S. (1982). Paradoxical sleep at selective times following training is necessary for learning. *Physiol Behav, 29*(3), 469-473.
Smith, C., & Lapp, L. (1986). Prolonged increases in both PS and number of REMS following a shuttle avoidance task. *Physiol Behav, 36*(6), 1053-1057.
Smith, C., & Rose, G. M. (1996). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiol Behav, 59*(1), 93-97.
Smith, C., & Rose, G. M. (1997). Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze. *Behav Neurosci, 111*(6), 1197-1204.
Smith, C., & Wong, P. T. (1991). Paradoxical sleep increases predict successful learning in a complex operant task. *Behav Neurosci, 105*(2), 282-288.
Smith, C., Young, J., & Young, W. (1980). Prolonged increases in paradoxical sleep during and after avoidance-task acquisition. *Sleep, 3*(1), 67-81.
Smith, C. T., Conway, J. M., & Rose, G. M. (1998). Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. *Neurobiol Learn Mem, 69*(2), 211-217.
Suchecki, D., Lobo, L. L., Hipolide, D. C., & Tufik, S. (1998). Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation. *J Sleep Res, 7*(4), 276-281.
Suchecki, D., Tiba, P. A., & Tufik, S. (2002). Hormonal and behavioural responses of paradoxical sleep-deprived rats to the elevated plus maze. *J Neuroendocrinol, 14*(7), 549-554.
Suchecki, D., & Tufik, S. (2000). Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat. *Physiol Behav, 68*(3), 309-316.
Sullivan, R. M., & Gratton, A. (2002). Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. *Brain Res, 927*(1), 69-79.
Tse, D., Langston, R. F., Kakeyama, M., Bethus, I., Spooner, P. A., Wood, E. R., et al. (2007). Schemas and memory consolidation. *Science, 316*(5821), 76-82.
Underwood, B. J. (1957). Interference and forgetting. *Psychol Rev, 64*(1), 49-60.
van Hulzen, Z. J., & Coenen, A. M. (1981). Paradoxical sleep deprivation and locomotor activity in rats. *Physiol Behav, 27*(4), 741-744.
van der Meulen, J. A., Bilbija, L., Joosten, R. N., de Bruin, J. P., & Feenstra, M. G. (2003). The NMDA-receptor antagonist MK-801 selectively disrupts reversal learning in rats. *Neuroreport, 14*(17), 2225-2228.
Wagner, U., Gais, S., Haider, H., Verleger, R., & Born, J. (2004). Sleep inspires insight. *Nature, 427*(6972), 352-355.

Wang, G. P., Huang, L. Q., Wu, H. J., Zhang, L., You, Z. D., & Zhao, Z. X. (2009). Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation. *Neuroreport, 20*(13), 1172-1176.

Whishaw, I. Q., & Tomie, J. (1997). Perseveration on place reversals in spatial swimming pool tasks: further evidence for place learning in hippocampal rats. *Hippocampus, 7*(4), 361-370.

Yoo, S. S., Hu, P. T., Gujar, N., Jolesz, F. A., & Walker, M. P. (2007). A deficit in the ability to form new human memories without sleep. *Nat Neurosci, 10*(3), 385-392.

Youngblood, B. D., Zhou, J., Smagin, G. N., Ryan, D. H., & Harris, R. B. (1997). Sleep deprivation by the "flower pot" technique and spatial reference memory. *Physiol Behav, 61*(2), 249-256.

Chapter 3

REM sleep deprivation using the inverted flowerpots method: high vs. low water level effects on sleep, recovery and learning

Abstract

The inverted flowerpot technique is typically the method used to administer rapid eye movement (REM) sleep deprivation in the literature investigating the role of REM sleep in learning. The impact of methodological variations in the technique on learning remains unclear. Although the number of pots and animals in the chamber and the number of hours in the chamber vary, one unaccounted for variable is the level of water within the deprivation chamber which is either 2 – 3 cm at the bottom of the chamber (low) or 1 – 3 cm from the top of the platform (high). The goal of my study was to determine the behavioral effects on learning following deprivation with either of these two levels of water within the deprivation chamber. I used 24 rats divided into 3 groups (controls, n = 7; REM sleep

deprived with low level water (LW), n = 10; and REM sleep deprived with high level water (HW), n = 7). Each rat performed 12 training trials per day in the Morris water maze, with additional probe trials at the start of the 4th and 6th days. Six hours of REM sleep deprivation was administered immediately following training during initial spatial learning on the first 3 days of the experiment. Starting from the 7th trial on the 4th day, rats were tested for subsequent reversal learning and were returned to their home cages following training each day. REM sleep deprivation with a high level of water (HW) did not lead to performance impairments during concurrent spatial learning or subsequent reversal learning when compared to controls. The level of retention was better for HW than LW for concurrent spatial learning. On the Day 6 probe trial, HW had a stronger preference for the reversal learning platform, while LW showed a stronger preference for the initial spatial learning platform location. Further, HW appeared more stressed than LW following the first day of REM sleep deprivation as they lost more weight after the manipulation. These results indicate that though HW appear initially more stressed than LW, LW appear less flexible with their learning as compare to HW. In addition, I measured (n = 4) the sleep / waking differences during 6 hrs of deprivation with both high and low levels of water, and the following post-deprivation period. Though REM sleep was eliminated in both groups, the expected REM sleep rebound was measured following deprivation only in the group with the low but not high level of water. This indicated that low water deprivation resulted in greater REM sleep pressure. These findings suggest that the general field of learning and REM sleep uses a more stressful

protocol for REM sleep deprivation. Furthermore, more rigorous attention to the deprivation protocol is required when comparing the findings across the learning and REM sleep deprivation literature.

Introduction

There have been a number of conflicting studies regarding the relationship between REM sleep deprivation (RD) and learning (for example: McGrath and Cohen 1978; Smith 1995; Hobson and Pace-Schott 2002; Vertes 2004; Rauchs, Desgranges et al. 2005; Stickgold and Walker 2005; Vertes and Siegel 2005). In general, both long and short bouts of RD (REM sleep restriction, rRS) have resulted in deficits in long-term potentiation (LTP, a physiological mechanism of learning) in the hippocampus (e.g. Davis, Harding et al. 2003; McDermott, LaHoste et al. 2003; Romcy-Pereira and Pavlides 2004; Kim, Mahmoud et al. 2005; Ishikawa, Kanayama et al. 2006; Ravassard, Pachoud et al. 2009) and spatial learning performance deficits (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Smith, Conway et al. 1998; Bjorness, Riley et al. 2005; Li, Tian et al. 2009; Wang, Huang et al. 2009). A comparison of the studies on RD and LTP revealed a short bout of RD resulted in prolonged impairments in LTP (Kim, Mahmoud et al. 2005), while one of the long duration RD studies resulted in relatively short lasting LTP impairments (Ravassard, Pachoud et al. 2009). Moreover, we recently observed that 6 hrs of RD did not result in the typically reported deficits in spatial learning (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Smith, Conway et al. 1998;

Bjorness, Riley et al. 2005; Li, Tian et al. 2009; Wang, Huang et al. 2009). The varying results observed following RD, may be the result of a previously uninvestigated, but key methodological difference (water level within the deprivation chamber) across these studies, as opposed to direct manifestations of the effect of RD itself.

All of the studies investigating the effects of RD on spatial learning, and the majority of the studies on the effects of RD on LTP have used the inverted flowerpot technique (Jouvet, Vimont et al. 1964) to administer RD. For this technique, an animal is placed on top of an inverted flowerpot, surrounded by water within a chamber. The platform of the inverted flowerpot is large enough for the animal to sit comfortably and enter quiet sleep, but small enough to prevent the animal from assuming a supported posture to enter REM sleep (RS) (Hicks, Okuda et al. 1977; McGrath and Cohen 1978). When the animal enters RS, the onset of muscle atonia results in the animal waking as they start to fall off the inverted flowerpot into the surrounding water.

A drawback of the inverted flowerpot technique for administering RD is the potential for increased stress, which can interfere with the interpretations on the effects of RD. To decrease potential stressors as a result of the methodology used, both the impact of social isolation and movement restriction have been tested while using the inverted flowerpot technique for deprivation. Stress induced by movement restriction can successfully be reduced when additional

inverted flowerpots are used as compared to just one within the RD chamber (van Hulzen and Coenen 1981). Attempts to reduce the effect of social isolation induced stress, by having multiple rats RS deprived within the same multiple platform chamber (Suchecki, Lobo et al. 1998; Suchecki and Tufik 2000; Suchecki, Tiba et al. 2002; Machado, Hipolide et al. 2004) have been less successful. This has been mostly due to issues with social hierarchy and dominance between group-housed rats. Additionally, the presence of additional rats moving around within the deprivation chamber can result in a rat having more frequent awakenings or disrupted sleep. The level of water within the deprivation chambers may alter the level of stress the rats undergo during the deprivation period. I hypothesized that a high level of water as opposed to a low level of water within the deprivation chamber may lead to more stressful conditions as the rat tries to maintain their body and tail out of the water.

It is currently unclear how a more stressful environment during RD can alter the recovery of RS (Rampin, Cespuglio et al. 1991; Rechtschaffen, Bergmann et al. 1999; Suchecki, Duarte Palma et al. 2000). However, stress as a potential interfering contaminant, when interpreting the effects of RD on behavior, was highlighted by Ruskin et al. (2006). Using adrenalectomized rats, Ruskin et al. (2006) measured the impact of RD on spatial working memory and spatial reference memory in the Morris water maze (72 hrs before prior to testing). Spatial reference memory is the retention of the platform position within the maze when entering from differing locations. Spatial working memory is recalling the

platform position when entering the maze from the same location as the trial immediately before. RD in the adrenalectomized rat resulted in impaired spatial working memory, while spatial reference memory was undisturbed. In contrast in an earlier study on the effects of RD on both spatial working and reference memory in the Morris water maze (24 hrs prior to testing, 24 hrs per day for the 4 days of testing) in the intact rat, a deficit in spatial reference memory but not spatial working memory was identified (Youngblood, Zhou et al. 1997). The comparison of these two studies suggests that RD in the absence of stress results in completely altered findings as compared to RD with uninhibited stress.

If one seeks to study the role of RS for learning, rather than the effects of stress itself, then these mixed results of stress on RD reinforce the necessity to minimize any potential stressors involved in the RD technique which may result from the RD chamber design. Contrary to what would be expected, most studies investigating the effects of RD on learning have continued to use a single platform, making it difficult to discern between the effects of RD versus the RD technique related stress on learning.

The specifics of the RD inverted flowerpot methodology used differ across both spatial learning and LTP studies, in the duration of RD, as well as in the height of the water, the number of inverted flowerpots and the number of rats within the deprivation chamber. Some studies have used three inverted flowerpots within the deprivation chamber and only 2 cm of water at the base of the chamber

(Chapter 2; Bjorness, Riley et al. 2005; Ravassard, Pachoud et al. 2009). In contrast, other studies used a level of water 1 - 2 cm from the top of the platform(s) with either a single inverted flowerpot in a chamber (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Davis, Harding et al. 2003; McDermott, LaHoste et al. 2003; Ruskin, Dunn et al. 2006) or multiple inverted flowerpots (McDermott, LaHoste et al. 2003; Li, Tian et al. 2009; Wang, Huang et al. 2009). Of those using multiple platforms, the number of rats within the deprivation chamber has differed between an individual rat (Chapter 2; McDermott, LaHoste et al. 2003; Bjorness, Riley et al. 2005; Ravassard, Pachoud et al. 2009) and multiple rats within one chamber (Li, Tian et al. 2009; Wang, Huang et al. 2009). Thus far, the effects of both the number of platforms and the number of rats within a single deprivation chamber have been assessed on the sleep / waking cycle (Suchecki, Duarte Palma et al. 2000; Machado, Hipolide et al. 2004; Machado, Suchecki et al. 2006), stress levels (van Hulzen and Coenen 1981; Suchecki, Lobo et al. 1998; Suchecki and Tufik 2000; Suchecki, Tiba et al. 2002). Additionally, the number of platforms used during RD has been assessed for the effect on LTP (McDermott, LaHoste et al. 2003). However, the impact of the differing level of water within the deprivation chamber has not been investigated. See Table 3.1 for a summary of the deprivation chamber protocols.

These results suggest that, of the aforementioned spatial learning behavioral or hippocampal-LTP studies (Smith and Rose 1996; Smith and Rose 1997;

Youngblood, Zhou et al. 1997; Smith, Conway et al. 1998; Davis, Harding et al. 2003; McDermott, LaHoste et al. 2003; Romcy-Pereira and Pavlides 2004; Bjorness, Riley et al. 2005; Kim, Mahmoud et al. 2005; Ishikawa, Kanayama et al. 2006; Li, Tian et al. 2009; Ravassard, Pachoud et al. 2009; Wang, Huang et al. 2009), the studies using three multiple inverted flowerpots with singly housed rats (Chapter 2; Bjorness, Riley et al. 2005; Ravassard, Pachoud et al. 2009) should have introduced the least amount of stress based on the previous literature on varying methodologies of the inverted flowerpot technique and the impact on stress in rats. I found no rRS-associated deficits in performance for concurrent spatial learning when I used a deprivation chamber with three inverted flowerpots and only a single rat (Chapter 2), as compared to prior experiments using a single inverted flowerpot with a high level of water (Smith and Rose 1997) or multiple flowerpots with a high level of water and multiple rats within the deprivation chamber (Li, Tian et al. 2009; Wang, Huang et al. 2009). Similarly, using multiple flowerpots and a low level of water, Ravassard et al. (2009) described shorter impairments of LTP as compared to others who used a single inverted flowerpot with a high level of water (Kim, Mahmoud et al. 2005) or multiple flowerpots and a high level of water (McDermott, LaHoste et al. 2003). It appears in comparing the results across these studies, the level of water within the deprivation chamber may lead to more pronounced experimental deficits. A potential reason for the water level within the deprivation chamber to have an effect on outcome measures is increased stress as a result of thermoregulation

issues with the rat's tail being in the water or less RS specific deprivation as the rat may need to maintain more muscle tone to keep their head above the water.

To date, there have been no comparative studies to address the potential differences as a result of water level used within the deprivation chambers. It is possible, that the potential differences in stress, sleep / waking characteristics while on the pots and subsequent rebound as a result of the variance in the water level, could have profound effects on behavioral outcomes of learning. Thus, in an effort to determine whether water level within the chamber could have given rise to the contrasting results between Smith and Rose's (1997) deficit in performance and my previous (Chapter 2) lack of performance deficit during spatial learning following rRS, we repeated the study from Chapter 2 using a high level of water within the RD chambers. We trained rats in the Morris water maze to test for the effects of rRS on initial spatial learning and subsequent reversal learning. To minimize the known side-effects of the inverted flowerpot RD technique, we used 3 inverted flowerpots and singly housed the rats within the RD chambers, while keeping the RD chambers alongside each other to limit isolation. We were then able to assess the effects of two different water levels during RD on learning. Overall, we expected the stress associated with high level water RD was a key contributing factor to previous findings described as RD effects. We expected similar effects to those seen with Smith and Rose (1996; Smith and Rose 1997) and amplification of the performance deficits seen during

subsequent reversal learning in my previous study (Chapter 2), with RD in the presence of high level water.

This study also facilitated examination of whether repeated days of RD with high level water had similar effects on a 'heavy load' of learning (12 trials) as on previously reported results with a 'lighter load' of learning (4 trials, Smith and Rose 1996) in the Morris water maze. As there was a later effect of low level water RD during spatial learning on subsequent reversal learning (Chapter 2), we chose to incorporate a 'reversal learning' component in this study to elucidate whether high level water RD during the initial spatial learning had similar effects on reversal learning. We hypothesized that RD with high level water causes a deficit in spatial learning performance in the Morris water maze task. We predicted that normal sleeping controls learn the Reversal Phase target location better than the high level water RS deprived group, based on previous findings with low level water within our lab (Chapter 2).

Methods

Animals

As described in Chapter 2, for all experiments, Sprague-Dawley male rats (~380 g; Harlan Indianapolis, IN) were used. Animals were housed in a 12:12 light cycle at an average temperature of 23°C. All procedures were approved by the animal

review board, the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. Rats had *ad libitum* access to fresh drinking water and food at all times except while in the water maze. Each rat was weighed at the start of each experimental day, before testing, to monitor changes in percent body weight.

REM sleep deprivation protocol

The general REM sleep deprivation protocol used in this study is the same as that described in Chapter 2. Three inverted flowerpots were placed into each of the deprivation chambers. Two levels of water within the REM sleep deprivation tank were used for this study: low and high. The low level had only 2 cm deep standing water at the base of the deprivation tank, ~22 cm below the base of the platforms which prevented the rats' tails from dangling in the water (Chapter 2; Bjorness, Riley et al. 2005; Ravassard, Pachoud et al. 2009). The high level water tank was filled until the water was 1 cm below the base of the platforms. This high level of water has been commonly used in a number of previous REM sleep deprivation and learning studies (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Smith, Conway et al. 1998; Beaulieu and Godbout 2000; Bjorness, Riley et al. 2005; Ruskin, Dunn et al. 2006; Li, Tian et al. 2009; Wang, Huang et al. 2009). Figure 3.1 shows a diagram of the two types of REM sleep deprivation tanks.

Experiment 1

Twenty four male Sprague-Dawley rats were tested for the effect of RD with high versus low levels of water on spatial learning using the Morris water maze.

Visual Water Maze protocol

The visual water maze protocol was described previously in Chapter 2.

Morris Water Maze protocol

Rats were randomly assigned into one of 3 learning groups: Controls (CON; n = 7), REM sleep deprivation with high level water following learning (HW; n = 7) and REM sleep deprivation with low level water following learning (LW; n = 10). Data for both CON and LW groups were previously reported in Chapter 2 as CONL and rRSL. Rats from HW were collected in conjunction with both LW and CON. The purpose of this chapter is to compare these data with the results of RD with high level water within the deprivation chamber following learning. Therefore, prior to testing, all rats used were naïve to the tasks. All rats performed the Morris water maze protocol previously described in Chapter 2. I used a 6 day protocol divided into two phases, the Learning Phase (Days 1, 2, 3, and first 6 trials on Day 4) and the Reversal Phase (starting from the 7^{th} trial on Day 4, and Days 5 and 6). Each day had training trials, with an additional probe trial at the start of Days 4 and 6. Once the animals were dry after training, I returned them to either their homecage or to the RD tanks. Both the HW and LW

groups were REM sleep deprived for the first 6 hrs immediately following training on Days 1, 2, and 3.

The dependent variables I measured were identical to those measured in Chapter 2. During learning trials the dependent variables measured were latency, pathlength, velocity and Gallagher's cumulative distance from the platform (Gallagher, Burwell et al. 1993). The latter variable measures the distance of the rat from the target platform at each second. Probe trial dependent variables measured were Gallagher's average proximity to the platform location, number of target platform location crossings, percent time in the target quadrant, pathlength and velocity.

Data Analyses and Statistics

The data analyses and statistical measures used for this study have been previously described in Chapter 2. In summary, trials were grouped into 2 consecutive trials forming trialsets (trials 1 - 3, 4 - 6, 7 - 9, and 10 – 12). I measured retention based on the difference in performance between the last trialset (trialset 4, trials 10-12) of one day and the first trialset (trials 1-3) the following day. To determine if there were initial differences in path chosen at the start of the trials, I measured the first 5 s of cumulative distance from the platform for training trials, and the first 10 s of average proximity to the platform location for the probe trials. In order to compare my data directly with the results from

Smith & Rose (1997) the average latency for the last 4 trials on day 1 was compared to the average latency for the first 4 trials on day 2.

Experiment 2

To determine the differences in sleep / waking characteristics with high level water versus low level water RD, 4 male, Sprague-Dawley rats were tested for the effect of high versus low levels of water in the deprivation chambers on the sleep cycle.

Surgery

Each animal was anesthetized with gaseous isoflurane then injected with a ketamine hydrochloride and xylazine hydrochloride mixture. The rat was determined to be sufficiently anesthetized when they did not respond to a toe-pinch, tested approximately 10 mins after injection. Once placed into the stereotaxic frame equipment, the skull was exposed and part of the neck muscle. Two wire hook electromyogram (EMG) electrodes were threaded through the nuchal muscles (AS636, Coone wire, CA) and 4 screw electroencephalogram (EEG) electrodes were placed into the skull: 2 bilaterally over the frontal cortex and 2 bilaterally over the parietal cortex (2 mm lateral, 2 mm anterior and posterior from Bregma). Four additional screws were placed into the skull as anchors. The electrodes were threaded through a 6-pin connector (Plastics One, Roanoake, NJ) and held in place using dental cement. Each rat was allowed 7

days to recover in individual housing. After 7 days of recovery, the rats were connected using tethers (Plastics One) to commutators (Plastics One) and allowed to habituate for 3 days. The commutators were connected via longer tethers to a data acquisition system (Neuralynx, Boseman, MT). Signals were recorded at a frequency of 666.7 Hz, with an analogue high pass filter of 1.0 Hz and a low pass filter of 125.0 Hz.

Sleep Recording Protocol

Two days after habituation to the recording system, rats were habituated to the deprivation chambers for 45 mins per day for two days (one day on the high level of water and the other on the low level of water). At time of testing, the EEG and EMG signals from the rats were recorded in their homecages for 24 hrs of baseline starting at lights on. The following day at lights on, rats were randomly assigned to tanks with either high or low level of water. After 6 hrs in the deprivation chamber, rats were returned to their homecages. Recordings continued for an additional 42 hrs. After the second day of recovery, a second period of baseline was recorded and the above protocol repeated, with each rat being placed into the tank with the alternative (high or low) water level.

Sleep / waking states were determined off-line using a within-lab designed sleep scoring program (Gross et al. 2009) based in MATLAB (2007b, Natick, MA). A state was scored when its criteria were met in at least 50% of the 10 s epoch. Active waking, quiet waking, quiet sleep, transitions to RS and RS were scored.

Active waking (AW) was scored when theta levels were high and EMG levels were high and modulated. Quiet waking (QW) was scored when EMG and theta levels were decreased but delta power was still relatively low. Quiet sleep (QS) was scored when delta power was high and EMG was further decreased. Transitions to RS (TR) were scored when sigma power (characterizing spindles) was high. RS was scored when theta power was high, delta / theta ratios were low and when EMG showed muscle atonia. All data were scored using a scorer blinded to the protocol. Sleep / waking characteristics were measured as total sleep time, percent time in wake (AW and QW), percent time in total sleep (QS, RS and TR), percent time of sleep spent in QS, percent time of sleep spent in RS, percent time of sleep spent in TR, latency to RS onset, number of wake bouts, number of RS bouts. In addition, we analyzed each of these measures normalized to percent baseline for each condition. Comparisons to baseline were within the same circadian periods – the 6 hrs on the platform starting at lights on were compared to the first 6 hrs after lights on the previous day during baseline recordings. The first 6 hrs post-deprivation were analyzed in 2 hr time windows following the return to their homecage. In addition, the post-deprivation period was analyzed as a total of 18 hrs, stopping at lights-on the following day. The first 6 hrs of lights-on that occurred 24 hrs after the start of the deprivation period was also analyzed and compared to the first 6 hrs of baseline. Figure 3.3 shows the protocol for this experiment.

Statistics

All analyses were done using SPSS (SPSS Inc. Chicago, IL). In all cases, when sphericity could not be assumed during a Repeated measures ANOVA (RMANOVA), the Huynh-Feldt correction was used.

In Experiment 2, RMANOVA were used to determine if there were differences resulting from water level during baseline recordings and recordings while on the pot or in the recovery phases. Dependent measures analyzed were percent of total recording time for waking and sleep and percent of total sleep for QS, RS and TR. Any differences that were identified in the RMANOVA were tested with post-hoc analyses of paired t-tests when necessary.

Results

Experiment 1 – the Effect of High level water REM sleep deprivation on Learning

This experiment was to determine the effects of REM sleep deprivation, with high level water following learning (HW) on spatial learning using the Morris water maze as compared to controls (CON). Based on my hypotheses and the current literature, I predicted that HW would have performance deficits as compared to CON. Further, this experiment was to identify if there were any prolonged effects of high level water RD during learning on subsequent reversal learning (see

Figure 3.3 for a review of the protocol). Lastly, I wanted to determine if high level water during RD versus a low level water during RD following learning (LW) yielded differing results. I predicted that HW would have poorer performance than LW, in particular during initial spatial learning.

As in my previous chapter (Chapter 2), I wished to thoroughly investigate the effects of rRS on spatial learning in the Morris water maze. To do this I used a number of variables to measure effects on training: latency to platform, pathlength, velocity and the Gallagher cumulative distance from platform. Probe trials were used to ascertain the level of learning using a range of variables: number of platform crossings, the Gallagher average proximity to the platform and percent time spent in target quadrant. Velocity and pathlength were also measured during the probe trials.

Experiment 1 - The Effects of High Level Water RD on Initial Spatial Learning

For all training trials, to allow for the change in platform location for the last 6 trials on Day 4, the trials for this study were divided into sets of 3 trials each. The learning trial data were analyzed across days (3 days, 4 trialsets per day), within day (Days 1, 2, and 3: 4 trialsets; Day 4: 2 trialsets) and within specific trialsets for each dependent variable.

Both groups, CON and HW, had performance improvements across the Learning Phase. For latency to platform, a group x day interaction was identified between HW and CON ($p = 0.039$; Figure 3.4), however when individual days were inspected, no group differences were found. No group differences or interactions were identified for either pathlength or cumulative distance to platform (Figure 3.5).

The level of retention was assessed to determine if rRS caused a 'resetting' or initial forgetfulness on the following day. For all variables measured during the training trials, the first trialset on Day 2 was subtracted from the last trialset on Day 1. Similarly the reset between Day 3 and Day 2 was measured. The reset between Day 4 and Day 3 could not be assessed due to potential interference from the probe trial at the start of Day 4. No group differences were found for reset on latency, pathlength or cumulative distance to platform, suggesting that when looking at trialsets, rRS did not interfere with retention the following day.

The first 5 s of each training trial was analyzed to determine if, at the start of the trial, either group took a more direct path to the platform as compared to the other group (Figure 3.6), this could be identified using the Gallagher cumulative distance measure. On the first trialset during Day 3, HW tended to perform better than CON ($p = 0.071$) however this did not reach significance throughout the Learning Phase.

These findings suggest that high level water RD immediately following training did not significantly affect latency, pathlength or Gallagher's cumulative distance measures.

There was a significant decrease in speed (velocity) across days ($p = 0.01$, linear fit; Figure 3.7). A day x trial interaction was also seen ($p < 0.001$, linear fit). HW had a slight tendency to swim faster than CON on the first trialset on Day 3 ($p = 0.096$). However, there were no significant group differences for training trials in the Learning Phase.

Experiment 1 - The Effects of High Level Water RD on the Day 4 Learning Phase Probe Trial

The probe trial on Day 4 was used as measure to identify differences between the two groups for the level of learning achieved. The variables were analyzed using the first 10 s and the entire 60 s separately. While no group differences were identified between HW and CON, all groups had a similar swim speed, a clear preference for the target quadrant, crossed through the platform area multiple times, had relatively low measures for the Gallagher's average proximity to the platform, and shared near equivalent pathlengths. These results continue to suggest that rRS immediately following 12 trials of spatial learning in the Morris water maze did not affect learning.

Previously published data by Smith & Rose (1997) reported significantly poorer performance in latency the next day following RD with high level water. In an effort to clearly compare my findings to the Smith and Rose (1997) study, I analyzed the last 4 trials of Day 1 compared to the first 4 trials on Day 2. In contrast to Smith et al.'s findings, no performance decrement was found on Day 2 following RD with high level water.

It is possible that the first 12 trials of learning on Day 1 in my study led to a learning plateau that was not reached in the Smith and Rose (1997) study on the first day. To address this, I compared the last trialset (3 trials) on Day 1 to the last trialset on Day 2. I found that there were no differences, suggesting that training performance was similar after 12 or 24 training trials.

Experiment 1 - Summary of the Effects of RD with High Level Water During Initial Spatial Learning

My results indicate that RD with high level water did not significantly alter performance on any of my variables measured during initial spatial learning.

Experiment 1 - The Effects of High Level Water RD on Subsequent Reversal Learning

To determine if RD with high level water during initial spatial learning could affect subsequent reversal learning, the platform location was changed to the opposite

side of the tank half-way through Day 4 and a further two days of training ensued. After each day of reversal training, all animals were immediately returned to their homecages. No sleep manipulations occurred during this phase of the experiment. The Reversal Phase training data were analyzed across days (2 days, 4 trialsets per day), within each day (Days 5, and 6: 4 trialsets; Day 4: 2 trialsets) and within specific trialsets.

Similar to the Learning Phase, performance for both groups improved across days and trialsets during the Reversal Phase, though no group differences were measured for latency (Figure 3.4), pathlength and cumulative distance to platform (Figure 3.5). Similar to the Learning Phase, retention on Day 5 was equivalent for both HW and CON for latency, pathlength and cumulative distance to platform.

When the first 5 s of each training trial was inspected to determine if there were initial group differences using the Gallagher measure (Figure 3.6), HW performed better across Day 4 reversal training than CON ($p = 0.047$). When looking at the individual trialsets, there was a trend for a group difference on the first reversal trialset on Day 4. On Day 5, performance was equivalent between the two groups. Retention between the end of Day 4 and the start of Day 5 was analyzed, but no group differences were found.

Swim speed decreased across reversal days ($p = 0.04$) and HW swam faster than CON across Days 5 and 6 ($p = 0.049$, Figure 3.7), with only a trend for a difference on Day 4 ($p = 0.095$).

Experiment 1 - The Effects of High Level Water RD on the Day 6 Probe Trial

To determine the level of learning on the Reversal Phase, the probe trial at the start of Day 6 was analyzed the first 10 s alone, and the entire trial length (60 s). When the first 10 s of the probe trial were analyzed, CON spent more time in the two target quadrants than HW ($p = 0.024$; Figure 3.8) and tended to have more target platform crossings in this time period too ($p = 0.084$; Figure 3.9). This would suggest that CON may have gone directly to the two target platform locations and initially had tighter search patterns than HW. When the individual Learning Phase and Reversal Phase platforms were analyzed on Day 6, no group differences were detected. When the entire trial length was considered, similar to the probe trial on Day 4, no group differences were identified for time spent in the target quadrant (Figure 3.10), number of platform crossings and the average proximity to the target platform (Figure 3.11).

Experiment 1 - Summary of the Effects of High Level Water RD on Subsequent Reversal Learning

Performance improved for both groups across training trials to find the reversed platform location, with HW swimming faster than CON.

Experiment 1 – Percent Body Weight

As a measure of stress, percent body weights were compared across the experiment. No group differences were found for percent body weight across the entire experiment or individual days, suggesting that high level water RD was not more stressful than normal sleeping conditions (Figure 3.12).

Experiment 1 - Summary of the effects of high level water RD on learning

Overall, although there were no differences on Day 1, throughout the experiment HW swam faster than CON ($p = 0.044$) and during the first 5 s of the trials, performed better on the cumulative distance to target platform than CON ($p = 0.02$) irrespective of the experimental phase. It appears that during the Day 6 probe trial, when looking at both target platform locations, CON had increased time spent in the two target quadrants and tended to have more platform crossings during the first 10 s of the probe trial as compared to HW. This suggests that HW did not initially swim with as great a preference for the two target platform locations during the probe trial. See Tables 3.2 A & B for results on the comparison between the effects of RD with high level water and normal sleeping controls on learning.

Experiment 1 - Comparison between RD with high level water and low level water on performance effects during initial spatial training

To determine the effect of water level within the deprivation chamber, performance was compared for rats RS deprived with high level water and rats RS deprived with low level water within the deprivation chambers.

As these results differed from those previously found with low level water RD, I compared my findings for high level water and low level water RD to determine how these groups differed. There were no performance differences during the Learning Phase between groups rRS with high level water and with low level water for latency, pathlength or cumulative distance from the platform when the entire trial was analyzed. Retention measured using the first 5 s of the trial for cumulative distance from the target platform between Days 2 and 3 showed a significant group difference where HW had better retention than LW ($p = 0.04$) with a trend for the same between Days 1 and 2 ($p = 0.082$) (Figure 3.6).

On Day 2 ($p = 0.04$) and the Learning Phase of Day 4 ($p = 0.018$), HW swam faster than LW (Figure 3.7)

Experiment 1 - Comparison between RD with high level water and low level water on performance effects during the Day 4 probe trial

There were no differences between HW and LW on the Day 4 probe trial.

Experiment 1 - Comparison between RD with high level water and low level water on performance effects during subsequent reversal training

On Day 4 reversal training, during the first 5 s of the trials (Figure 3.6), HW had significantly better measures for the cumulative distance to platform as compared to LW ($p = 0.003$). This continued through the first trialset on Day 5, but not beyond. No other group differences were identified for latency, pathlength or cumulative distance from the platform during the Reversal Phase.

HW tended to swim faster than LW ($p = 0.054$) across the Reversal Phase (Figure 3.7).

Experiment 1 - Comparison between RD with high level water and low level water on performance effects during the Day 6 probe trial

On Day 6, LW spent significantly more time in the Learning Phase target quadrant ($p = 0.031$, Figure 3.10) than HW, while HW showed a stronger preference for the Reversal Phase target location with a lower average proximity measure than LW ($p = 0.002$, Figure 3.11). The average proximity to platform also showed a platform x group interaction ($p = 0.002$), with LW tending more towards the Learning Phase platform on Day 6 and HW tending more towards the Reversal Phase platform. Overall, HW learned the two target locations better than LW as measured by the average proximity to the target platform ($p = 0.001$).

Experiment 1 – Summary of the comparisons between RD with high level water and low level water on learning

During the Learning Phase, HW had significantly better retention than LW at the start of the trials. HW also swam faster than LW. During the early parts of the Reversal Phase, HW swam more towards the Reversal Phase platform at the start of the trials than LW. HW continued to tend to swim faster than LW. On the Day 6 probe trial, prior low level water RD resulted in a preference for the Learning Phase platform location, while high level water RD resulted in a preference for the Reversal Phase platform location. This latter finding is similar to findings previously reported between normal sleeping controls and animals RD with low level water (Chapter 2). See Tables 3.3 A & B for results in the comparison between the effects of high and low level water RD on learning.

Experiment 1 - Comparison between RD with high level water and low level water on the change in percent body weight

One possible difference between the two RS deprived groups is the level of stress. I measured stress by differences in percent body weight. On Day 2 of the experiment, there was a significant difference between the two RS deprived groups ($p = 0.03$) when HW lost more percent body weight, while LW had no loss (Figure 3.12). This would suggest that after the first RD period, HW may have been more stressed than LW.

Experiment 1 – Summary of the effects of water level during RD on performance

I found that RD with high level water did not alter performance during initial spatial learning or subsequent reversal learning as compared to controls. HW did, however swim faster though did not differ from controls in changes in percent body weight.

In the comparison of high level water versus low level water RD, HW had better initial retention during initial spatial learning and during subsequent reversal learning had better cumulative distances measures at the start of the trials than LW. HW had stronger preference for the Reversal Phase platform location, with LW continuing to prefer the Learning Phase platform location on the Day 6 probe trial. HW tended to swim faster than LW, and lost significantly more percent body weight after the first bout of RD as compared to LW.

Experiment 2 – Comparison of the effects of RD with either low water level and high water level RD on sleep / waking characteristics

In an effort to determine whether the difference in water levels during RD gave rise to changes in the sleep / waking architecture, independent of learning, I recorded EEG and EMG of rats RS deprived with both high level water and low level water. I expected that low level water RD would result in more RS specific deprivation than high level water RD.

Experiment 2 - Sleep / waking characteristics during and following a 6 hr deprivation period

To determine the effects of high level water (S-HW) versus low level water (S-LW) within the RD chambers, on the sleep / waking characteristics, both during the deprivation period and following. The data were divided into sections for analysis (see Figure 3.3 for a review). While comparisons for both water levels were made with their own baselines, no differences were identified between the baseline for S-LW and the S-HW baseline.

Experiment 2 - Comparison of the sleep / waking characteristics during the RD and baseline periods

During S-LW RD, there was a significant decrease in total time spent in sleep ($p = 0.033$) as compared to baseline. In contrast, deprivation with S-HW only showed a trend for a decrease in total time spent in sleep ($p = 0.087$) as compared to baseline. There were no differences in the number of waking episodes with either water level.

Total time spent in quiet sleep (QS; Figure 3.13), REM sleep (RS; Figure 3.14) and transition-to-REM sleep (TR; Figure 3.14) were calculated as a percent of total time in sleep. A significant increase in QS was found with both S-LW ($p = 0.003$) and S-HW ($p = 0.024$), however when QS was measured as a percent of total recording time (waking + sleep), there were no significant differences in QS for either water level. Differences in QS can largely effect measurements in

percent time in sleep as it is a substantial component. The significant change in QS was only detectable as a percent time of sleep but not when waking was also considered. A complete loss of RS was detected across the 6 hrs of deprivation ($p = 0.015$ for S-LW; $p = 0.017$ for S-HW). There was a trend for S-LW to have more TR than S-HW ($p = 0.075$) during the deprivation period. However, while I expected TR deprivation with S-HW when compared to baseline, it was unexpectedly only observed for S-LW compared to baseline ($p = 0.028$), and not for S-HW. It appears that although S-LW did have slightly more TR than S-HW, the significant deprivation in TR for S-LW is a result of higher levels of TR during the S-LW baseline. Overall, both water levels caused complete RS deprivation, with S-LW appearing less specific, depriving TR as well.

Experiment 2 - Comparison of the sleep / waking characteristics during the post-deprivation and baseline periods

The first 2 hrs immediately following deprivation showed a significant increase in QS ($p = 0.003$) and a decrease in RS ($p = 0.045$) compared to baseline. However, the increase in QS was not detected when measured as a percent of total sleep / waking recording time. No differences were found in the sleep / waking measures specific to either water level.

Between hrs 2 and 4 post-deprivation, total time spent in sleep was equivalent for both conditions. With no differences between the QS baselines, S-LW deprivation resulted in a trend for less QS as compared to its baseline ($p =$

0.093), and to S-HW deprivation (p = 0.081; Figure 3.15). S-LW had a significant increase in RS compared to baseline (p = 0.049), which was not observed for S-HW (Figure 3.16). No differences in TR were found for either water level.

In the following 2 hr period (4 – 6 hrs post-deprivation), no differences were detected in total time spent in sleep. No further RS rebound for S-HW or S-LW was identified, although there was a trend for a decrease in TR for S-LW (p = 0.052) that was not seen for S-HW. The TR means were similar for both baseline and recovery between the two groups, though the variability was much higher for the S-HW than the S-LW baseline.

Across the whole 18 hrs immediately following RD, a RMANOVA suggested a trend for increased total time in sleep (p = 0.069), which reached significance following deprivation with S-LW (p = 0.029). There were no group differences in time spent in RS, QS or TR. Latency to REM onset was significantly longer following S-HW compared to baseline (p = 0.016). The baseline for S-LW was highly variable due to one rat not entering REM sleep for a protracted length of time (251.6 min). When this animal was removed from the dataset, the results were not significantly altered, therefore the animal was kept in the dataset.

Lastly, I investigated whether there were any differences in sleep / waking characteristics during the first 6 hrs of the next lights-on period (hrs 18-24) following deprivation. This period also coincided with the circadian time of the

deprivation period itself (see Figure 3.3). Overall this post-deprivation period had a significant increase in total time in sleep ($p = 0.009$), with trends for increased RS ($p = 0.063$, Figure 3.8 B) with an increase in number of REM sleep episodes ($p = 0.066$) and a decreased number of waking bouts ($p = 0.089$). This trend for a decrease in number of waking bouts was retained by S-HW deprivation ($p = 0.086$). S-LW deprivation produced a trend for a decrease in TR ($p = 0.072$), which was not seen for the S-HW deprivation condition. The same TR baseline for the RD period is being used here, where the TR baselines between the two groups were not equivalent. The TR levels between the S-HW and S-LW for the 18-24 hr period are fairly equal, suggesting no real differences of TR between these groups at this time period.

Experiment 2 - Summary of sleep and sleep rebound results for HW Vs LW RD techniques.

RD with both water levels resulted in RS deprivation. S-LW had a deprivation in TR as compared to their baseline, though tended to maintain more TR when compared to S-HW during the deprivation period. The post-deprivation data suggest an increase in REM sleep pressure following S-LW deprivation, which was alleviated during the second 2 hr post-deprivation window. Overall, RS amounts were not different between the two water levels within 18 hrs following deprivation, suggesting that the S-HW group recovered the difference in RS slowly across the night. Further, fluctuations in TR were observed for S-LW and not for S-HW.

Discussion

My hypotheses were several fold. I hypothesized 1) RD with high level water would result in a performance deficit on Day 2 of spatial learning; and 2) RD with high level water would result in a performance deficit in reversal learning as compared to controls. Surprisingly, both these hypotheses were disproved, with high level water RD resulting in better learning of the Reversal Phase platform location than low level water RD. Tables 3.2 A & B and 3.3 A & B show a summary of the behavioral results from the Morris water maze dataset. I also found that S-LW had a larger RS rebound effect for sleep homeostasis than S-HW.

REM sleep restriction effects on initial spatial learning and subsequent reversal learning

The previous studies that have reported that RD following learning results in a deficit in performance have reported on latency to platform (Smith and Rose 1996; Smith and Rose 1997; Li, Tian et al. 2009; Wang, Huang et al. 2009), pathlength (Li, Tian et al. 2009; Wang, Huang et al. 2009), number of quadrant entries during training (Smith and Rose 1996), area under the curve for both latency and pathlength (Youngblood, Zhou et al. 1997) and the percent time spent in target quadrant during a probe trial (Wang, Huang et al. 2009). Along

with my previous study (Chapter 2), this is one of the first studies to use the more sensitive Gallagher measures when studying the effects of rRS.

In a previous study from our lab (Chapter 2) I reported that RD with low level water during learning did not result in a performance deficit during initial spatial learning but instead resulted in an impairment of reversal learning as compared to normal sleeping controls. Together, with my current study, I clearly did not find the impairment during initial spatial learning associated with rRS that has been previously reported (Smith and Rose 1997). During the Reversal Phase, when sleeping normally, rats previously RS deprived with high level water swam faster than controls, which could be a sign of either increased urgency to find the platform or increased mobility as a result of prior movement restriction. The increased speed can not be solely due to the movement restriction of RD, as both the low level water RD group and the high level water RD group were restricted to three inverted flowerpots. To further this, the HW group swam significantly faster than LW on Day 2, which was also the day that HW had greater percent body weight loss as compared to LW. Overall, as there were no differences in pathlength or latency to platform associated with the increases in swim speed for HW when compared with either CON or LW, the differences in velocity were therefore not substantial enough to affect these correlative measures. Though this also indicates that the increase in swim speed was ineffective for locating the platform faster.

Based on the performance differences between CON and HW, one could posture that RD with a high level of water could lead to increased flexibility or malleability of learning. The better performance of HW as compared to CON at the start of reversal learning on Day 4 suggests that the HW group were able to learn that a new target location existed faster or was more open to an alternative platform location existing. Additionally, on Day 6, both groups had equivalent performance in the first 10 s of the probe, however when both previously targeted platform locations did not have a platform, HW looked elsewhere while CON remained searching within the two previous platform locations (CON had greater percent time in the combined target locations on Day 6).

During spatial learning, the HW group had better retention between the end of Day 2 and the start of Day 3 than LW did. Curiously, if timed from the first bout of RD, this difference in retention falls near the 48 hr window previously associated with a deficit in LTP resulting from 4 hrs of RD by gentle handling (Romcy-Pereira and Pavlides 2004). The difference in retention, along with the finding that HW seemed to learn both target locations better than LW (Day 4 learning platform and Day 6 reversal platform) indicates that low level water RD impaired general learning as compared to high level water RD. Lastly, HW performed better than LW during the first three trialsets of reversal learning and had a preference for the reversal platform location on the Day 6 probe as compared to LW, who preferred the location of the initial Learning Phase platform location on Day 6. These two findings indicated that low level water RD resulted in a more

fixed, less flexible learning pattern, where LW would hold onto the initial platform location they were learning while manipulated. This resulted in LW taking more trials to learn the new platform location, while remaining ‘tied’ to the old platform location.

An alternative explanation is that RD with a high level of water following training learning aided learning by the deprivation water level mimicking a similar environment to the Morris water maze.

Another possible explanation for the differences in performance between the two groups could result from a difference in strategies being used. For HW to learn the new location on the reversed trials faster than LW, and even as compared to CON, HW may be more reliant on procedural strategies. These would enable the HW group to locate a new platform faster as they learned how to do the task rather than the definite location of the platform. An rRS-associated switch from using hippocampal-dependent strategies has been previously reported (Bjorness, Riley et al. 2005). For groups more reliant on spatial mapping strategies, it would take theoretically take them longer to learn to repeatedly search elsewhere. The difference in strategies utilized by the groups can also be supported with the Day 6 probe, where HW searches elsewhere once the platform is not located in the previous locations, while for example CON, that may have been more dependent on spatial mapping strategies, remained fixed to the two previous locations. The difference in strategies utilized would not account though for the previously

reported difference between low level water RD and CON during reversal learning (Chapter 2).

The combined results may suggest that both low level water RD and CON groups utilize a spatial learning strategy, while high level water RD uses procedural strategies. Further, LW's spatial learning may be impaired or restricted in later learning (e.g. reversal learning) as compared to CON. The difference in stress, between the two rRS groups, associated with the first day of rRS following training may have been sufficient to diverge the two rRS groups into two different modes of learning.

LTP has been shown to be impaired in the hippocampus with increased levels of stress (Foy, Stanton et al. 1987). Therefore, an impaired hippocampus to learn spatial mapping could force a rat to use procedural strategies more related on other brain structures such as the basal ganglia.

One potential reason why rats RD with high level water in my study may not have shown deficits similar to previous studies, is that all rats were first exposed to two days of visual platform maze. This would have introduced all rats to some of the non-hippocampal dependent strategies (Morris 1984; Morris, Hagan et al. 1986) for the task prior to the first day of spatial learning and rRS.

Interestingly while neither RS deprived groups differ to CON during the Learning Phase, the two RS deprived groups did differ from each other. Therefore, the level of the water within the chambers is more effective at altering performance than RS deprivation itself. Further, although it has been previously reported that RS deprivation during learning can lead to a deficit in subsequent reversal learning (Chapter 2) it is now difficult to determine if this effect was a result of RS deprivation or a different factor. I propose, however, that RD with low level water provides a more accurate account of the effects of RD, while high level water RD is contaminated with stress or other factors. I here utilize changes in percent body weight as a correlate of stress, where decreases in percent body weight are associated with increased stress levels.

Van Hulzen and Coenen (1981) showed that the multiple platform inverted flowerpot technique was less stressful (determined by changes in rat weight) than the single platform method, suggesting that the previous studies on learning, in the Morris water maze, and RD by the platform method were more stressful than my current protocol. Therefore my results could be seen as a more accurate or less contaminated dataset with respect to stress. In my study, HW had a higher percent body weight loss after the first day of testing as compared to LW, but afterwards had comparable percent body weights to the other two groups (CON and LW). This would suggest that although the rats were habituated to the RD chambers with the two levels of water prior to testing, that the high level water RD was still more stressful than low level water RD, but only

for the first day. Therefore, based on body weight change as a sign of stress, the water level in the RD chamber should be kept at a low level to reduce the effect of stress interfering with interpretations of the effect of RD itself.

My results indicated that there were no effects of rRS on latency measures between days 1 and 2. Irregardless of using similar Morris water maze paradigms, my results are in strong contrast to Smith and Rose's work (1997). Although, the high level of water did not result in a measurable increase in stress as compared to CON, the contrasting results between our lab and Smith and Rose's previous work (1997) could still be linked to stress related to movement restriction, as previously described. Other protocol differences between these studies to consider are the duration of RD and rat strain. Our lab administered RD for 6 hrs as compared to 4 hrs (Smith and Rose 1997). Although Smith and Rose (1997) did not provide sleep / waking measurements, our multiple platform RD technique resulted in complete RD for both levels of water, suggesting that unless a rebound effect within the initial 2 hr period following RD in the Smith and Rose (1997) study resulted in the poorer performance in their RS deprived group, my increased RD period should not have recovered the performance I measured. While the sources for the rats were different, both groups used Sprague Dawley adult male rats. With no probe trial, it is difficult to determine an accurate difference in learning between controls and RS deprived groups in the Smith and Rose (1997) study.

With similar loads of learning on the Poe 8-box maze (Bjorness, Riley et al. 2005) and the 8 – arm maze (Smith, Conway et al. 1998), administering rRS across their multiple day studies, both Bjorness et al. (2005) and Smith et al. (1998) found performance deficits in spatial learning throughout large portions if not the entire length of their study. In contrast Smith and Rose (1996) who performed a similar Morris water maze protocol to my current study but with only 4 training trials per day, only found an rRS-associated performance deficit at the start of Day 2 of a 4 day experiment. This observed deficit at the start of Day 2, was found in both Morris water maze studies by Smith and Rose (1996; 1997) independent of the load of learning, 4 training trials or 12 training trials per day. This suggests that in the Morris water maze, the learning load itself should not influence the delay to when the rRS deficit should be observed, unless a maximal threshold of learning was reached within the first day of testing. In looking at my data, 12 trials in our Morris water maze may have brought the rats to asymptotic learning within the first day, where there was no difference between performance (latency, pathlength or cumulative distance) at the end of Day 1 and the end of Day 2. It would be difficult for rRS to have a modulatory effect if a ceiling effect was already reached.

Sleep / waking recordings

To determine whether the exposure to the two different water levels while being RS deprived may have greatly altered the sleep / waking characteristcs, I

measured EEG and EMG during baseline, 6 hrs of RD and the subsequent 24 hrs.

My study showed that for the 6 hr period in the RD chambers, with three inverted flowerpots, both water levels resulted in complete elimination of RS. Low level water also resulted in an overall decrease in total sleep and a decrease in transitions to RS during RD. S-LW and not S-HW had a RS rebound, measured during the 2-4 hr post-RD period. In fact, S-HW had a delayed RS onset following RD. S-LW also had an overall increase in total sleep time across the first 18 hrs post-RD period.

Few others regularly report on TR, although a similar state in humans has been linked with learning (Nishida and Walker 2007). Although statistically, S-LW had a TR decrease compared to their baseline while S-HW did not, the baseline of the S-HW group was lower than the S-LW group to begin with. I found that TR amounts tended to be higher for S-LW than S-HW during the deprivation period, which would further suggest that the S-HW situation suppressed TR. The difference between the two groups during the deprivation period suggests that S-LW could be more specific for RS deprivation than S-HW, which had lower TR during deprivation as compared to S-LW.

In a recent study using an identical RD chamber as the S-LW group, (Mashour, Lipinski et al. in review) found a sizeable RS rebound within the first 4 hrs of the

post – RD period following 24 hrs of RD. This RS rebound was not detectable later in the recovery phase. A similar result was also found by Ravassard et al. (2009), following 72 hrs of RD there was an increase in RS within approximately the first three hours of the post – RD period. To test for stress, they measured corticosterone levels, and found that low level RD did not result in an increase in corticosterone levels as compared to controls. They also found that there was a significant drop in corticosterone levels for rebounding rats as compared to controls. This drop in corticosterone levels would roughly overlap with the period of RS rebound observed in my study.

RD for this study was performed at lights-on, which is a time period generally associated with relatively low quantities of RS. Therefore the amount of RS lost was fairly mild, although complete. The beginning of the lights-on period was chosen to coincide with the period that would be affected with the behavioral experiment, where testing started at lights-on and the 6 hr RD followed immediately afterwards. If S-HW and S-LW were RS deprived later in the lights-on cycle, there may have been a larger or more differentiated response to the RD techniques than I observed.

With the lower level water, it was expected that rats would be less stressed and would have decreased muscle tone since they are not trying to keep their tails and head from hanging over the side of the pots into the water as would happen with the high level water. S-LW RD compared to its baseline did show a

deprivation in TR, which could be an indicator of more successful RD (commented on in Fujihara, Serino et al. 2003). This is supported by the RS rebound seen only for S-LW. The lack of RS rebound and the delayed RS onset for S-HW RD could indicate that S-HW was more physically stressful (Cui, Li et al. 2007) than S-LW. This would coincide with the drop in percent body weight following high level water RD for the HW group that wasn't seen for LW, as physical stress is also associated with a loss of body weight. Alternatively, however, the RS rebound in S-LW and lack of a change in body weight for the LW group could be a result of psychological stress (Cui, Li et al. 2007). Although during RD with low level water, the rats are only elevated 22 cm from the surface of the water it may be sufficiently high to induce increased anxiety levels. Of the two possibilities, the results for the low level water are less likely the result of increased psychological stress as compared to the results for the high level water being associated with increased physical stress.

Although I found some differences in sleep / waking characteristics between high level and low level water during RD, it seems unlikely that they could result in the performance differences I observed in the Morris water maze. While increases in RS can be correlated with improvements in learning (e.g. Smith and Wong 1991), this does not necessarily link with RS rebound. In fact, Li et al. (2009) and Wang et al. (2009) have conflicting results, where release from extensive RD, which would result in RS rebound, in one case did improve the impaired performance, while the other did not. The RS rebound observed for the S-LW, as compared to

S-HW, 2-4 hrs post-RD could have led to a difference in performance. The increase in RS rebound would be expected to benefit performance. However, because of the mixed results for TR during RD, it would be too speculative to comment on how this may have contributed to the performance differences between the two groups.

Summary

My study is the one of the first to investigate the relationship with rRS and spatial Morris water maze learning to such an extent. I failed to find an effect of rRS on initial learning with high level water RD. The performance related differences between the rats RS deprived with high level water as compared to rats RS deprived with low level water raises the concern that previously reported deficits in spatial learning resulting from RD (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Li, Tian et al. 2009; Wang, Huang et al. 2009), may indeed have not been a result of RD, but related to contaminants of the technique such as stress. If the effects of rRS were more robust than RD technique-related effects, I would have expected rats RS deprived with high level water to be no better in performance than rats RS deprived with low level water. Further, based on previous work in our lab (as described in Chapter 2), I would have also expected reversal learning impairments for HW as compared to controls like I found in the low level water RD rats. Future studies are required to compare the effects of RD, with multiple platforms versus single platforms for individually housed rats, on spatial learning. This will help discern between the

effects of RD and contaminants related to the inverted flowerpot method for RD. In addition, future studies are necessary to determine if rRS has an affect on the consolidation of a lighter learning load (e.g. 4 trials per day) in the Morris water maze as measured by a wider range of variables, and a probe trial as described here. My study suggests that low level water should be used to further minimize stress when using the inverted flowerpot RD technique, and care taken to consider the mode of RD when describing the effects of RD or rRS on learning.

Table 3.1 Summary of the deprivation chambers used in learning and REM sleep studies

	In the deprivation chamber		
Reference	**number of platforms**	**number of rats**	**height of water**
Bjorness et al., 2005	3	1	low
Davis et al., 2003	1	1	high
Li et al., 2009	14	multiple	high
McDermott et al., 2003	1	1	high
McDermott et al., 2003	5	1	high
Ravassard et al., 2009	3	1	low
Ruskin et al., 2006	1	1	mid
Smith and Rose, 1996	1	1	high
Smith and Rose, 1997	1	1	high
Walsh dissertation, Chapter 2	3	1	low
Wang et al., 2009	14	multiple	high
Youngblood et al., 1997	1	1	high

The number of platforms, rats and the height of water in a single deprivation chamber are described for the studies listed.

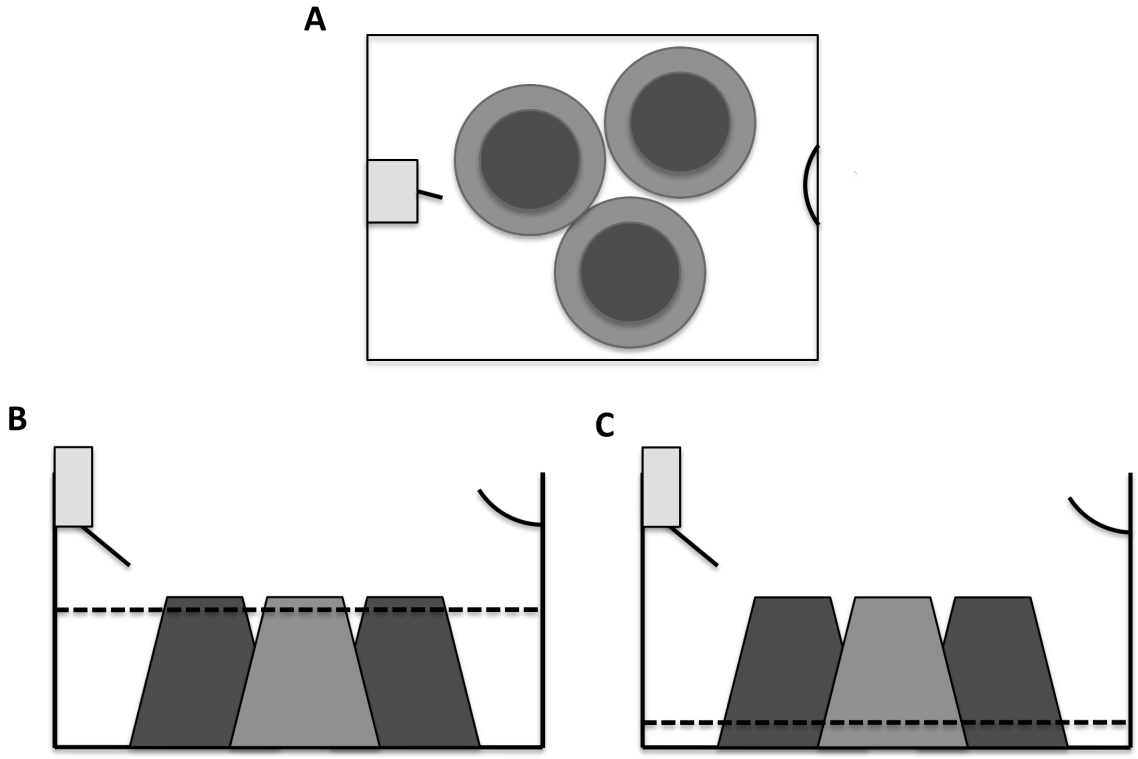


Figure 3.1 Depiction of the REM sleep deprivation chambers
A) Overview of the Deprivation chambers. To the left of the image is the easily accessible water bottle, to the right is the food trough. In the center of the chamber are three inverted flowerpots. Figures B and C depict cross-sectional images of the Deprivation chambers. B) The HW level water, where the chambers are filled with water to within 1 cm of the inverted flowerpot platforms. C) The LW level water, where the chamber is filled with 2 cm of water surrounding the inverted flowerpots. Note these images are a depiction and are not drawn to scale.

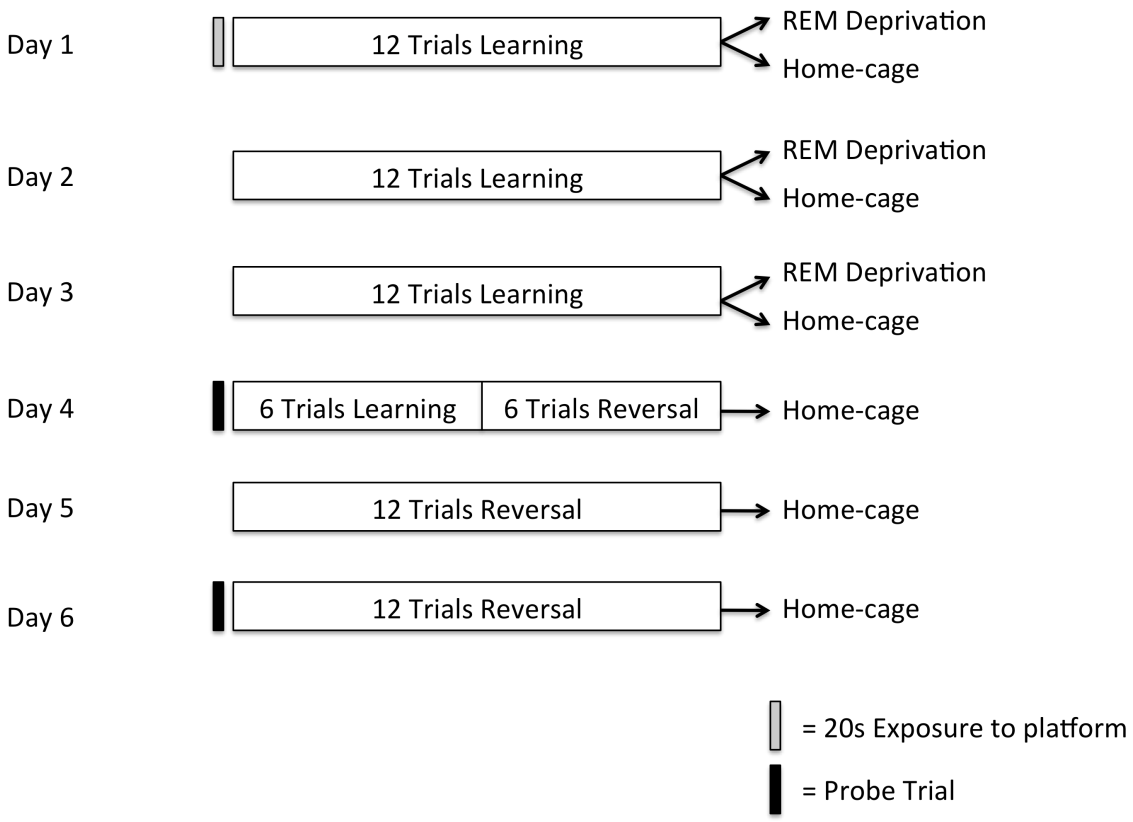


Figure 3.2: Experiment 1 protocol
There are 6 days within the protocol. Each day had 12 trials, with an additional probe trial on Days 4 and 6. At the start of Day 1, the rats were placed on the hidden platform for 20 s. From the 7th trial Day 4 onwards, the training trials changed from learning trials to reversal trials, when the platform was placed in the opposite quadrant as compared to the learning trials. Following training on days 1, 2, and 3, the HW and LW REM deprivation groups underwent 6 hrs of REM deprivation immediately after the water maze. Controls were returned to their homecages, as were all rats on days 4 and 5. Probe trials are indicated as solid black rectangles. The initial habituation 20 s period is indicated as a solid grey rectangle.

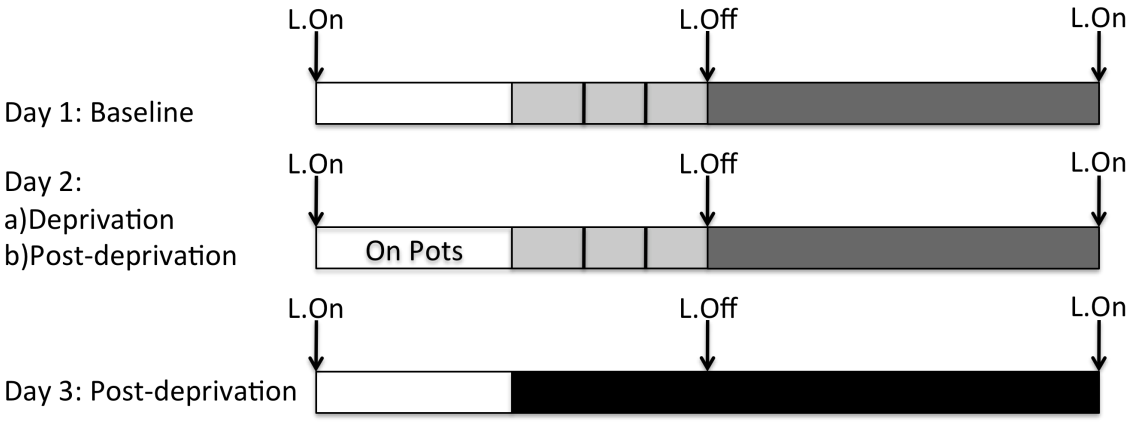


Figure 3.3 - Experiment 2 protocol
There were three periods of recording per water level: baseline, time on the flower- pots (On Pots) and post-deprivation. The first 6 hrs (white) of lights on (L.On) on Day 1 – baseline, were compared to Day 2 A – deprivation, and to Day 3 – post-deprivation. The following 18 hrs of Day 1 baseline and Day 2 post-deprivation were compared to each other (grey). Further, the last 6 hrs of lights on were divided into 2 hr time windows and compared on Day 1 baseline and Day 2 post-deprivation. The procedure and comparisons made were identical for both HW and LW level water.

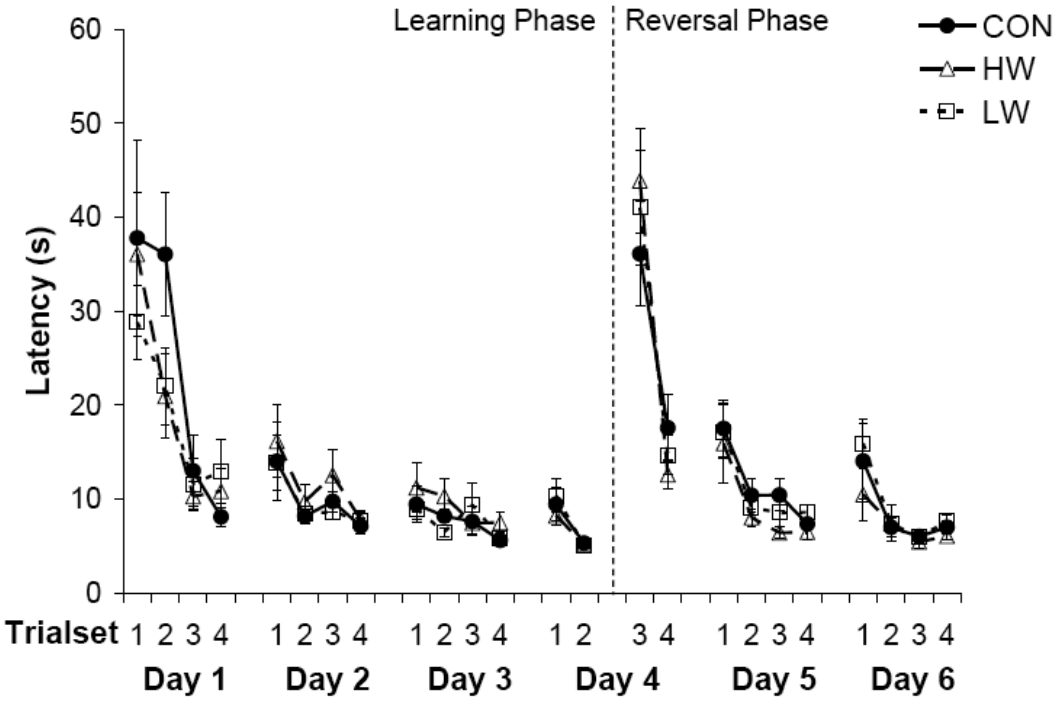


Figure 3.4 Latency to platform
Latency to platform is shown for both the Learning Phase and the Reversal Phase for controls (CON, filled circle), rats REM sleep deprived with high level water (HW, open triangle) and rats REM sleep deprived with low level water (LW, open square). Data are shown as mean ± SEM. No differences between groups were found for these measures.

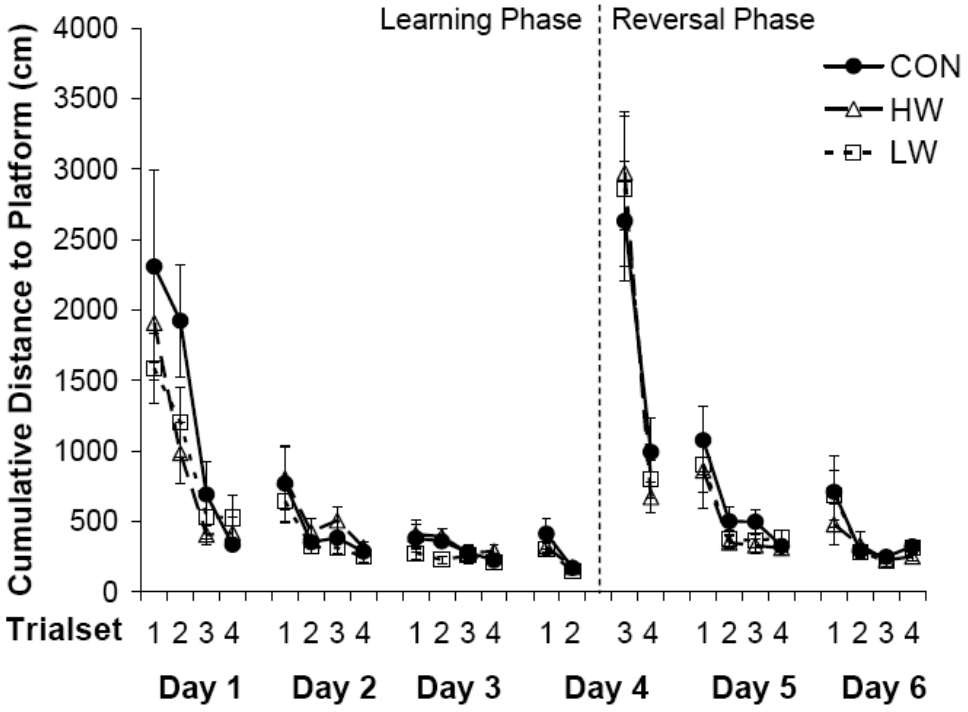


Figure 3.5 – Cumulative distance from the platform
Cumulative distance from the platform is shown for both the Learning Phase and the Reversal Phase for controls (CON, filled circle), rats REM sleep deprived with high level water (HW, open triangle) and rats REM sleep deprived with low level water (LW, open square). Data are shown as mean ± SEM. No differences between groups were found for these measures.

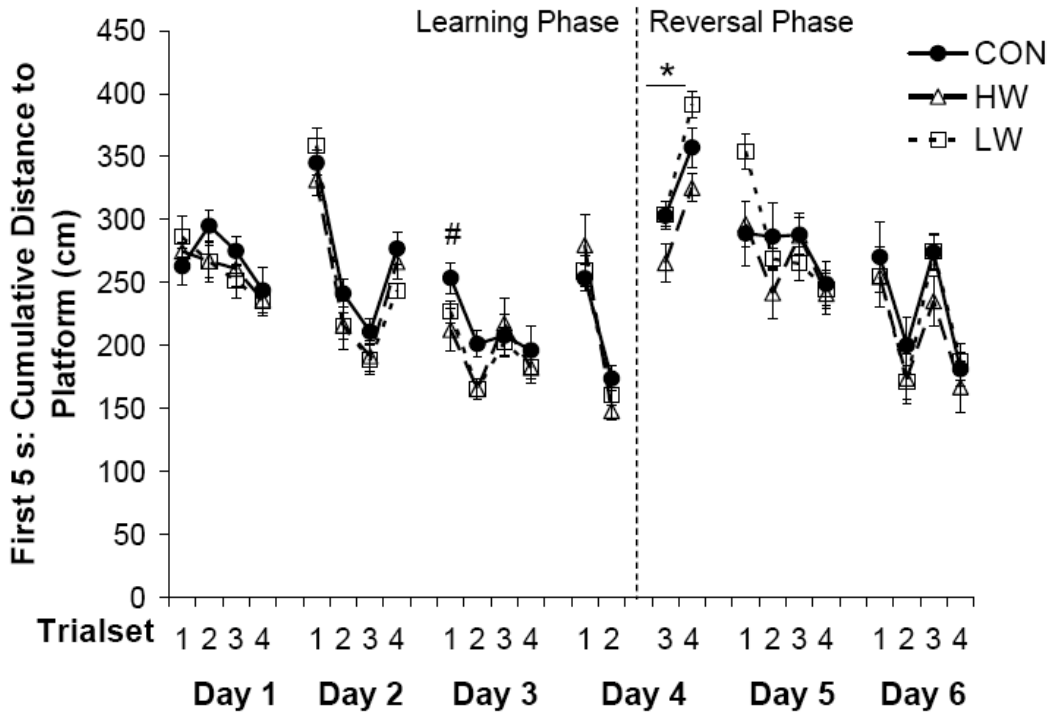


Figure 3.6 First 5 s of cumulative distance from the platform
Cumulative distance from the platform for the first 5 s of the trials is shown for both the Learning Phase and the Reversal Phase for controls (CON, filled circle), rats REM sleep deprived with high level water (HW, open triangle) and rats REM sleep deprived with low level water (LW, open square). Data are shown as mean ± SEM. rRS was administered after training on Days 1, 2 and 3. HW tended to swim closer to the Learning Phase platform location on the 1st trialset on Day 3, and significantly closer to the Reversal Phase platform location on Day 4 trialsets 3 and 4 than CON. * $p < 0.05$. # $p < 0.1$.

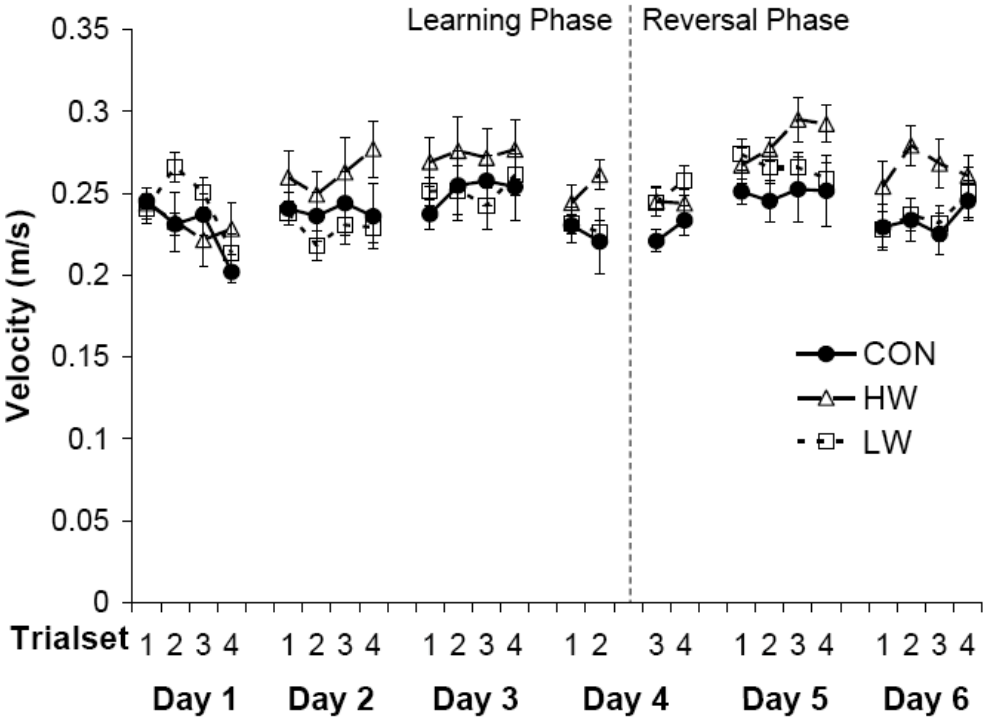


Figure 3.7 Velocity
Velocity is shown for both the Learning Phase and the Reversal Phase for controls (CON, filled circle), rats REM sleep deprived with high level water (HW, open triangle) and rats REM sleep deprived with low level water (LW, open square). Data are shown as mean ± SEM. Group differences were identified, where HW swam faster than both controls and LW.

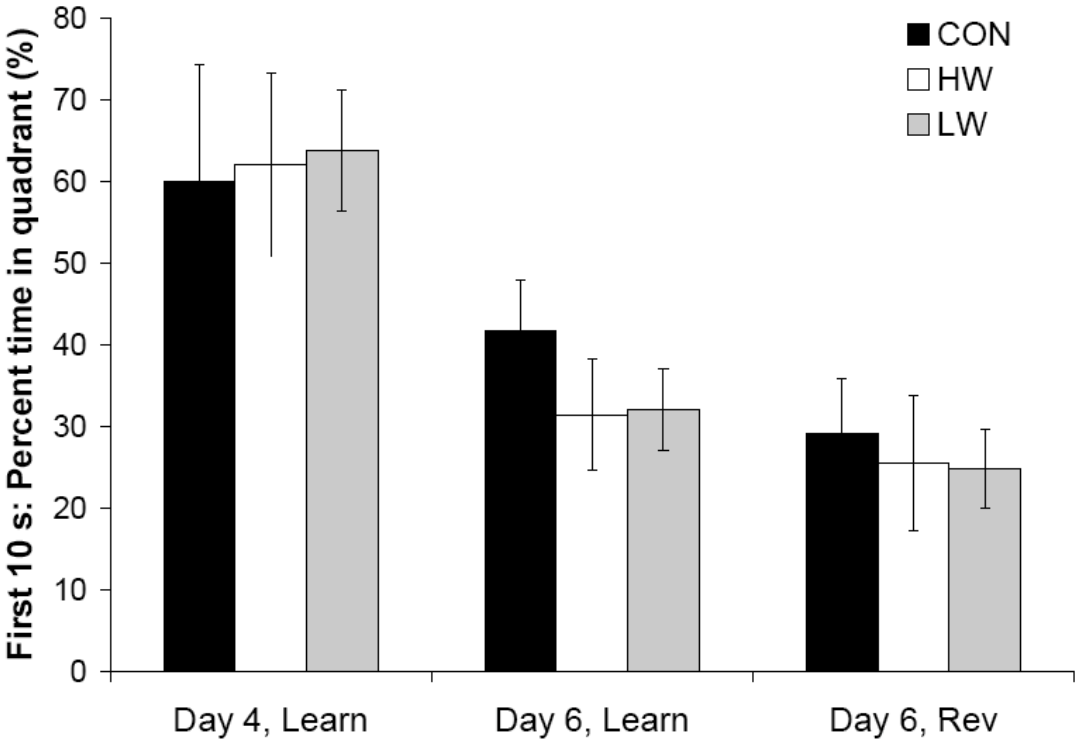


Figure 3.8 Percent time spent in quadrant within the first 10 s of the probe trials

The percent time spent in the target quadrant for the first 10 s of the probe trials are shown for the Day 4 probe trial for the Learning Phase (Learn) platform location, and both the Learning Phase (Learn) and the Reversal Phase (Rev) platform locations on the Day 6 probe trial. Data are shown as mean ±SEM for CON (black), HW (white) and LW (grey).

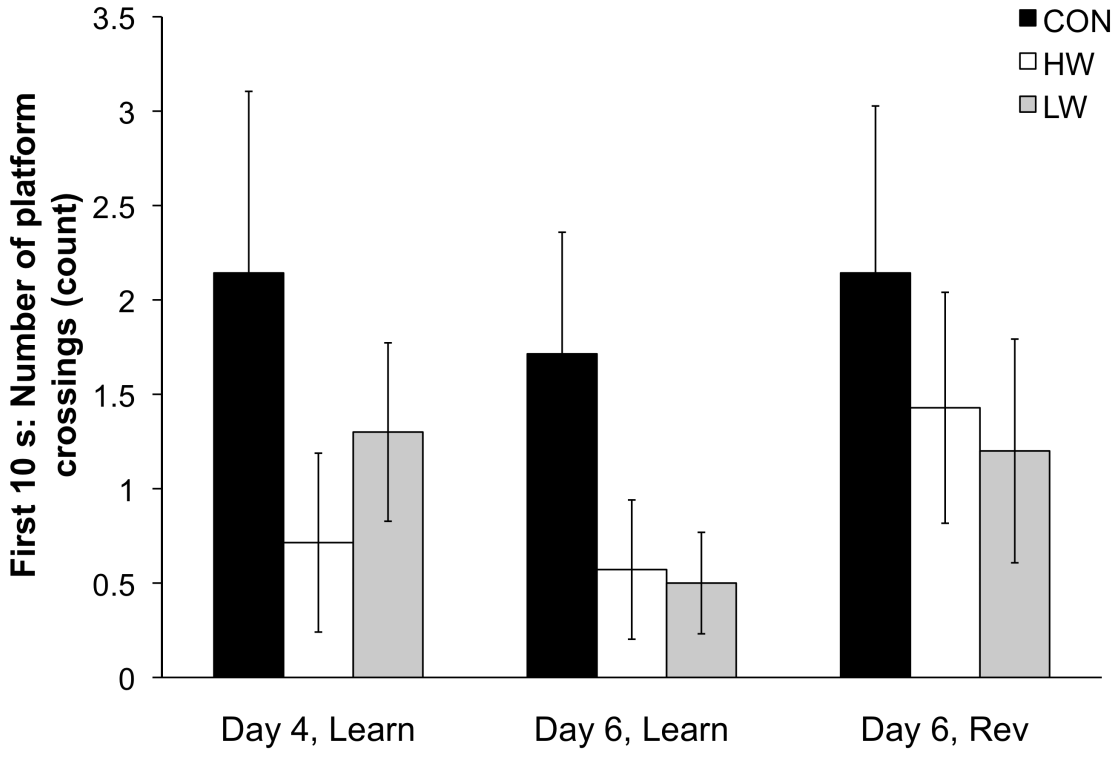


Figure 3.9 Number of Platform Crossings within the first 10 s of the probe tests

The number of platform crossings are depicted for the first 10 s of the probe trials are shown for the Day 4 probe trial for the Learning Phase (Learn) platform location, and both the Learning Phase (Learn) and the Reversal Phase (Rev) platform locations on the Day 6 probe trial. Data are shown as mean ± SEM for CON (black), HW (white) and LW (grey).

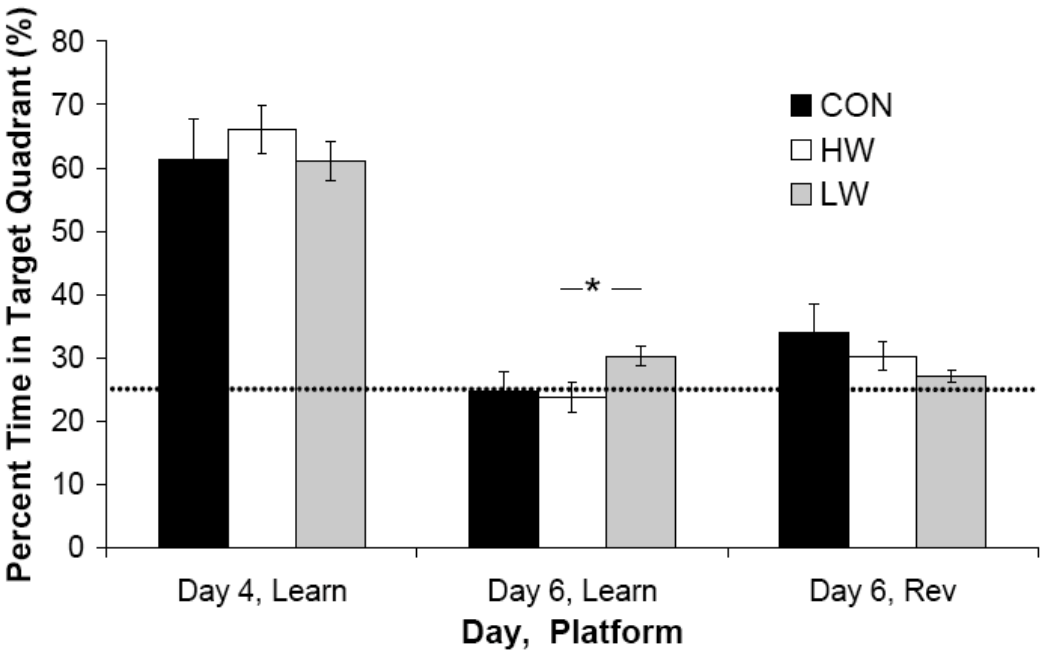


Figure 3.10 Percent time spent in the target quadrant during the 60 s Probe trial

Percent time spent in the target quadrant for the entire 60 s probe trial for the Day 4 Learning Phase (Day 4, Learn), Day 6 Learning Phase (Day 6, Learn) and the Day 6 Reversal Phase (Day 6, Rev) is shown as mean ± SEM for CON (black), HW (white) and LW (grey). LW spent more time in the Learning Phase quadrant on the Day 6 probe trial than HW. The dashed line indicates chance (25 %). The LW group spent significantly more time in the Learning Phase quadrant on the Day 6 probe trial as compared to the HW group. * $p < 0.05$

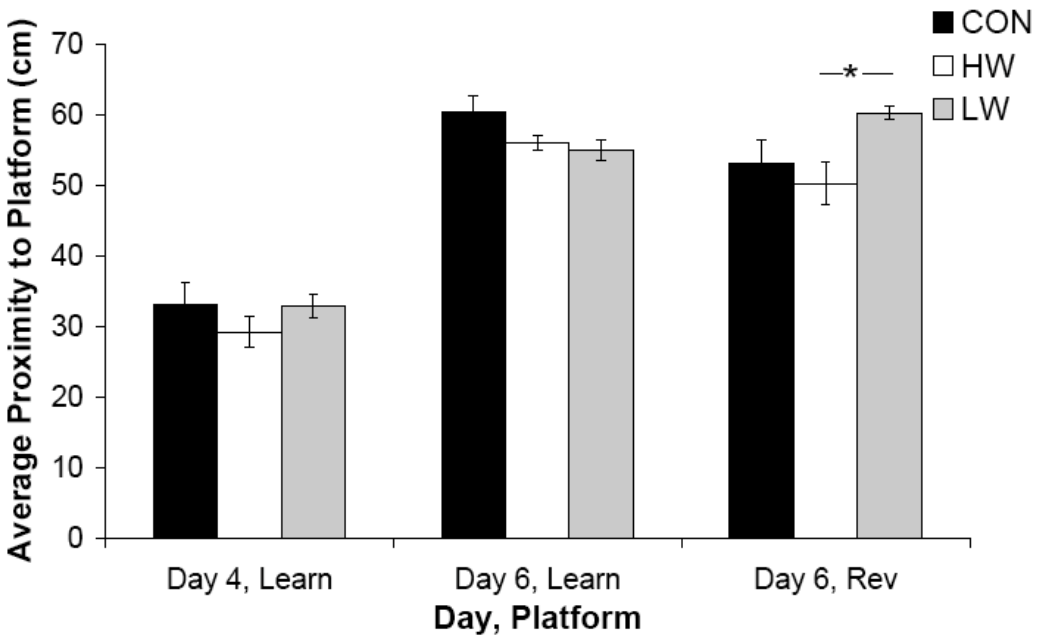


Figure 3.11 Average proximity to platform during the 60 s Probe trial
Average proximity to the platform location for the entire 60 s probe trial for the Day 4 Learning Phase (Day 4, Learn), Day 6 Learning Phase (Day 6, Learn) and the Day 6 Reversal Phase (Day 6, Rev) is shown as mean ± SEM for CON (black), HW (white) and LW (grey). HW swam closer to the Reversal Phase platform location on the Day 6 probe trial than LW. * $p < 0.05$

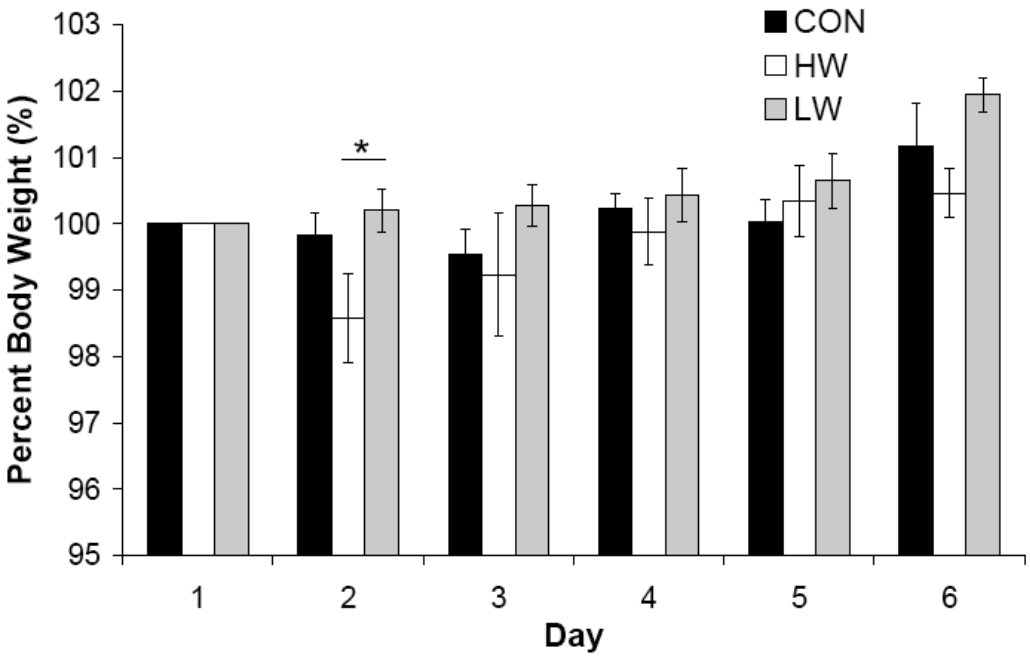


Figure 3.12 Percent Body Weight
Percent body weights for the experiment are shown as mean ± SEM for CON (black), HW (white) and LW (grey). HW and CON did not differ in percent body weight. HW had a greater loss of percent body weight on Day 2 as compared to LW. * $p < 0.05$

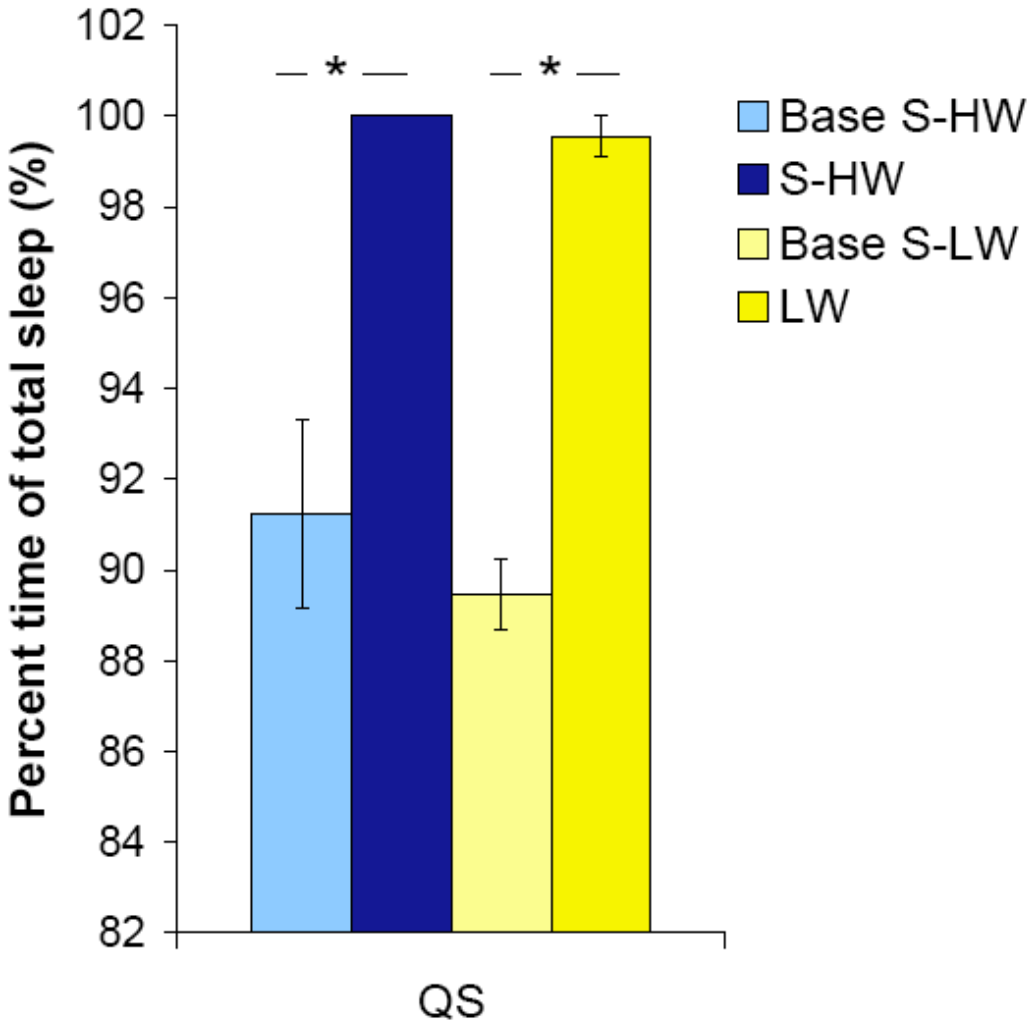


Figure 3.13 Quiet sleep during the 6 hr deprivation period
Sleep recordings are shown for the 6 hrs in the deprivation chambers S-LW (yellow) and S-HW (dark blue), and the corresponding baseline periods (Base S-LW (pale yellow) and Base S-HW (light blue)). Data are shown as mean ± SEM for quiet sleep (QS) as percent time of total sleep.

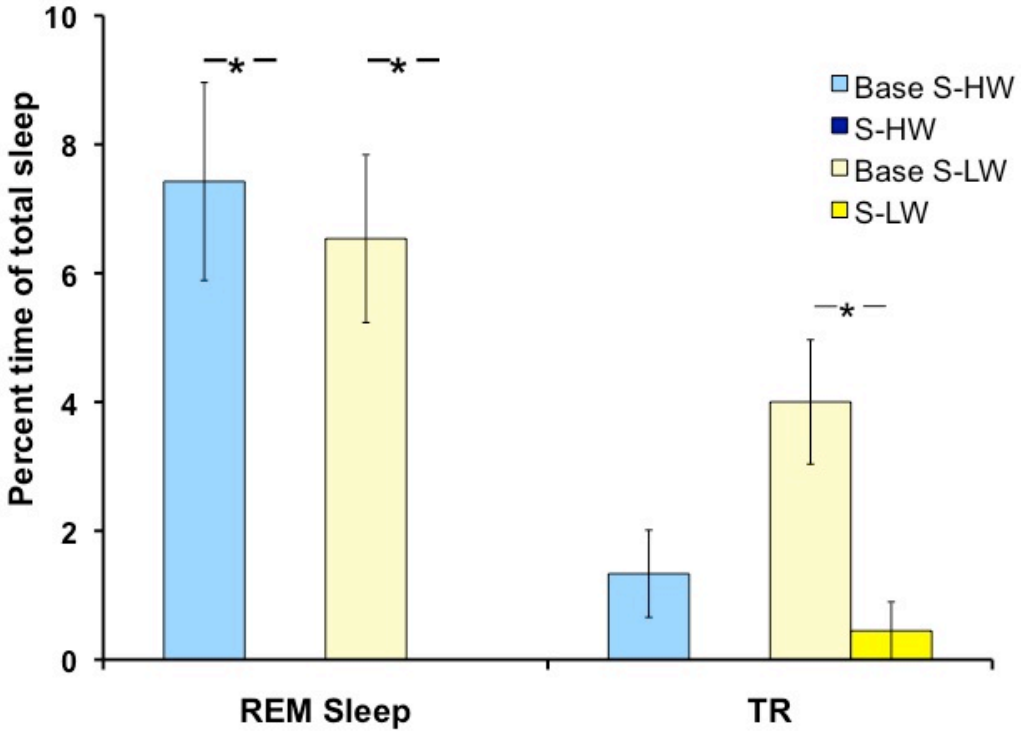


Figure 3.14 REM sleep and transitions to REM sleep during the 6 hr deprivation

Period Sleep recordings for REM sleep (RS) and Transitions to REM sleep (TR) as a percent of total sleep for the 6 hrs in the deprivation chambers S-LW (yellow) and S-HW (white), and the corresponding period of baseline (Base S-LW (pale yellow) and Base S-HW (light blue)) are shown as mean ± SEM. * $p < 0.05$.

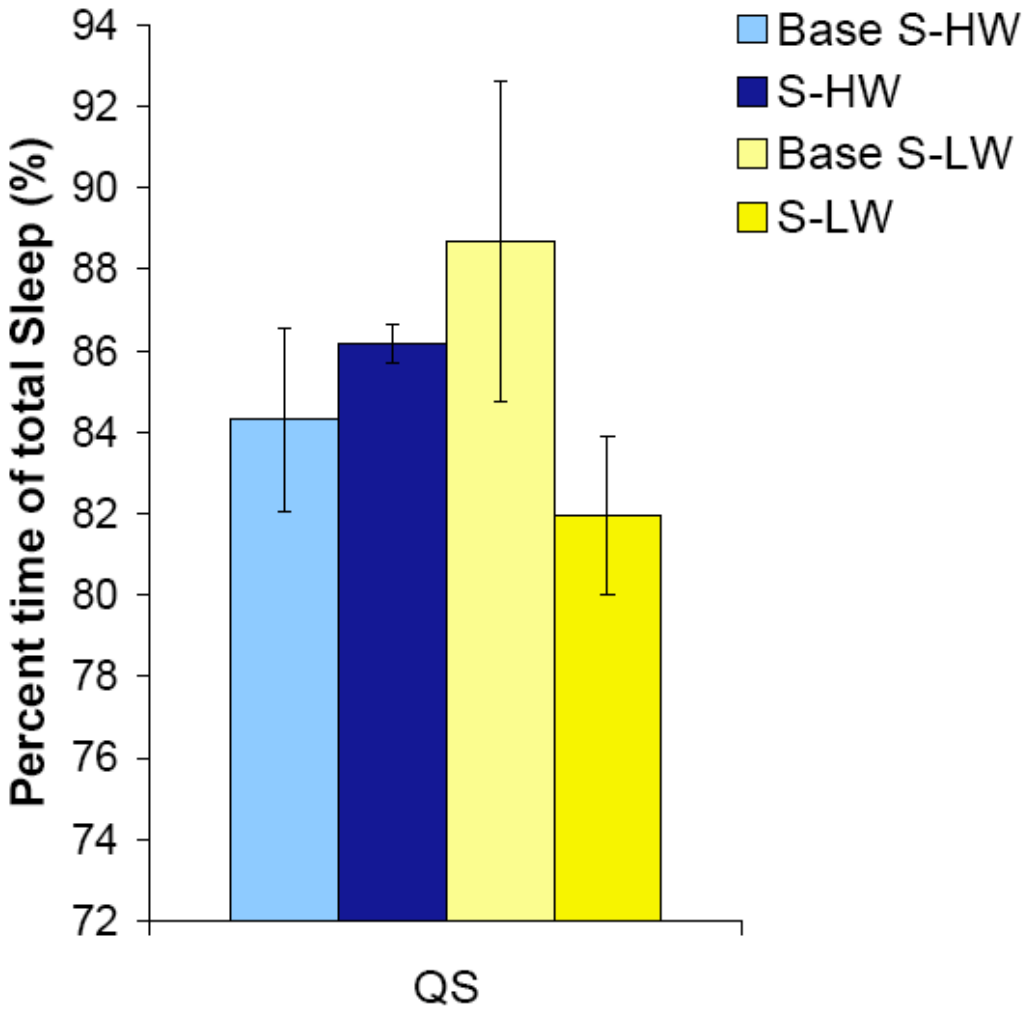


Figure 3.15 Quiet sleep during the 2-4hr post-deprivation period
Sleep recordings are shown for the 2 – 4 hr window post-deprivation period for S-LW (yellow) and S-HW (dark blue), and the corresponding baseline periods (Base S-LW (pale yellow) and Base S-HW (light blue)) for the percent time of total sleep spent in quiet sleep (QS). Data are shown as mean ± SEM.

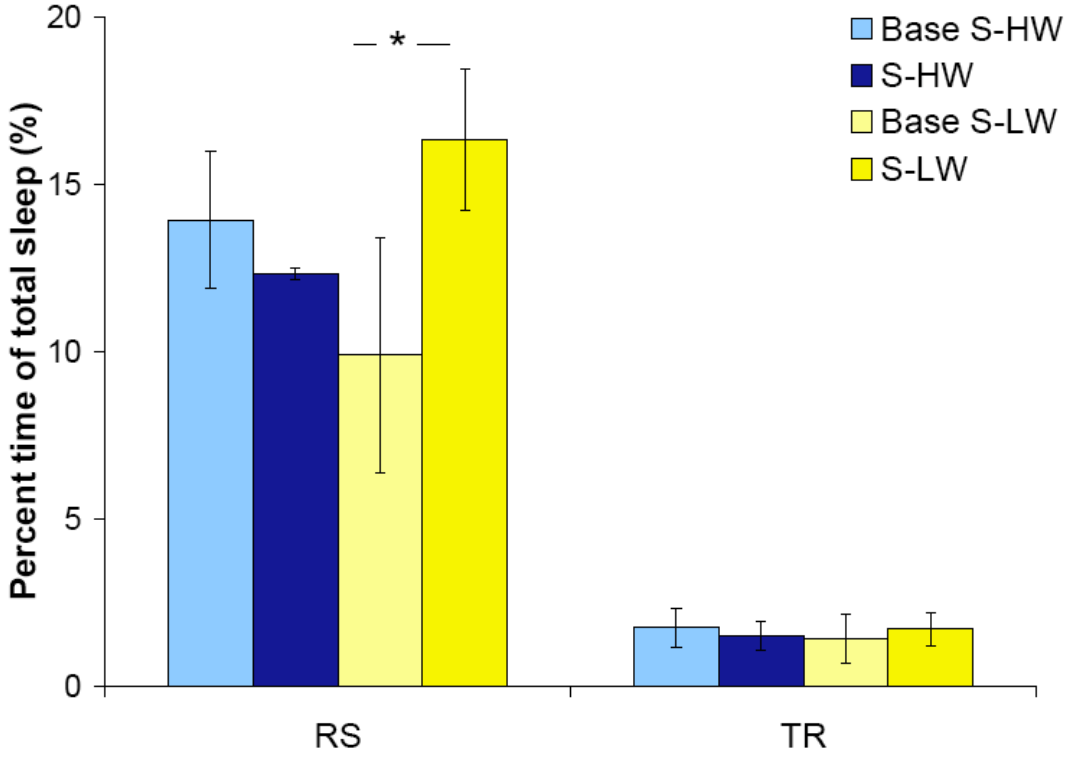


Figure 3.16 REM sleep and transitions to REM sleep during the 2-4hr post-deprivation period

Sleep recordings are shown for the 2 – 4 hr window post-deprivation period for S-LW (yellow) and S-HW (dark blue), and the corresponding baseline periods (Base S-LW (pale yellow) and Base S-HW (light blue)) for the percent time of total sleep spent in both REM sleep (RS) and transitions to REM sleep (TR). Data are shown as mean ± SEM. * $p < 0.05$.

Table 3.2 A Summary of performance differences resulting from RD with high level water as compared to controls during the Learning Phase

	HW
Training	Learning Phase
Latency	-
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	# Day 3 trialset 1: better than CON
Swim Speed	# faster than CON
Probe Trial	
60 s Percent time in Learning Phase Quadrant	-
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	-
60 s Avg. Proximity to Reversal Phase Platform	-
10 s Probe Percent time in target quadrant	-
10 s Number of platform crossings	-
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

Table 3.2 B Summary of performance differences resulting from prior RD with high level water as compared to controls on the subsequent Reversal Phase performance

	HW
Training	Reversal Phase
Latency	-
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	* Day 4: better than CON
Swim Speed	* Day 5: faster than CON
Probe Trial	
60 s Percent time in Learning Phase Quadrant	-
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	-
60 s Avg. Proximity to Reversal Phase Platform	-
10 s Probe Percent time in target quadrant	* (L.P. & R.P.) less time than CON
10 s Number of platform crossings	# (L.P. & R.P.) fewer crossings than CON
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

L.P. Learning Phase, R.P. Reversal Phase. * $p < 0.05$. # $p < 0.1$

Table 3.3 A Summary of performance differences resulting from RD with high level water as compared to low level water during the Learning Phase

	HW Vs. LW
Training	Learning Phase
Latency	-
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	* Recall: HW better than LW
Swim Speed	* HW faster than LW
Probe Trial	
60 s Percent time in Learning Phase Quadrant	-
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	-
60 s Avg. Proximity to Reversal Phase Platform	-
10 s Probe Percent time in target quadrant	-
10 s Number of platform crossings	-
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

Table 3.3 B Summary of performance differences resulting from prior RD with highlevel water as compared to low water level on the subsequent Reversal Phase performance

	HW Vs. LW
Training	Reversal Phase
Latency	-
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	* Day 4: HW better than LW
Swim Speed	# HW faster than LW
Probe Trial	
60 s Percent time in Learning Phase Quadrant	* HW spent less time than LW
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	-
60 s Avg. Proximity to Reversal Phase Platform	* HW swam closer than LW
10 s Probe Percent time in target quadrant	
10 s Number of platform crossings	
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

* $p < 0.05$. # $p < 0.1$

References

Beaulieu, I. and R. Godbout (2000). "Spatial learning on the Morris Water Maze Test after a short-term paradoxical sleep deprivation in the rat." Brain Cogn **43**(1-3): 27-31.

Bjorness, T. E., B. T. Riley, et al. (2005). "REM restriction persistently alters strategy used to solve a spatial task." Learn Mem **12**(3): 352-359.

Cui, R., B. Li, et al. (2007). "Differential effects of psychological and physical stress on the sleep pattern in rats." Acta Med Okayama **61**(6): 319-327.

Davis, C. J., J. W. Harding, et al. (2003). "REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus." Brain Res **973**(2): 293-297.

Foy, M. R., M. E. Stanton, et al. (1987). "Behavioral stress impairs long-term potentiation in rodent hippocampus." Behav Neural Biol **48**(1): 138-149.

Fujihara, H., R. Serino, et al. (2003). "Six-hour selective REM sleep deprivation increases the expression of the galanin gene in the hypothalamus of rats." Brain Res Mol Brain Res **119**(2): 152-159.

Gallagher, M., R. Burwell, et al. (1993). "Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze." Behav Neurosci **107**(4): 618-626.

Gross, B. A., Walsh, C. M., Turakhia, A. A., Booth, V., Mashour, G. A., & Poe, G. R. (2009). Open-source logic-based automated sleep scoring software using electrophysiological recordings in rats. *J Neurosci Methods, 184*(1), 10-18.

Hicks, R. A., A. Okuda, et al. (1977). "Depriving rats of REM sleep: the identification of a methodological problem." Am J Psychol **90**(1): 95-102.

Hobson, J. A. and E. F. Pace-Schott (2002). "The cognitive neuroscience of sleep: neuronal systems, consciousness and learning." Nat Rev Neurosci **3**(9): 679-693.

Ishikawa, A., Y. Kanayama, et al. (2006). "Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus." Eur J Neurosci **24**(1): 243-248.

Jouvet, D., P. Vimont, et al. (1964). "[Study of Selective Deprivation of the Paradoxal Phase of Sleep in the Cat.]." J Physiol (Paris) **56**: 381.

Kim, E. Y., G. S. Mahmoud, et al. (2005). "REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus." Neurosci Lett **388**(3): 163-167.

Li, S., Y. Tian, et al. (2009). "The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats." Learn Behav **37**(3): 246-253.

Machado, R. B., D. C. Hipolide, et al. (2004). "Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery." Brain Res **1004**(1-2): 45-51.

Machado, R. B., D. Suchecki, et al. (2006). "Comparison of the sleep pattern throughout a protocol of chronic sleep restriction induced by two methods of paradoxical sleep deprivation." Brain Res Bull **70**(3): 213-220.

Mashour, G. A., W. J. Lipinski, et al. (in review). "Isoflurane Anesthesia is not Permissive of Homesotatic Processes related to Rapid Eye Movement Sleep."
McDermott, C. M., G. J. LaHoste, et al. (2003). "Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons." J Neurosci **23**(29): 9687-9695.
McGrath, M. J. and D. B. Cohen (1978). "REM sleep facilitation of adaptive waking behavior: a review of the literature." Psychol Bull **85**(1): 24-57.
Morris, R. (1984). "Developments of a water-maze procedure for studying spatial learning in the rat." J Neurosci Methods **11**(1): 47-60.
Morris, R. G., J. J. Hagan, et al. (1986). "Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function." Q J Exp Psychol B **38**(4): 365-395.
Nishida, M. and M. P. Walker (2007). "Daytime naps, motor memory consolidation and regionally specific sleep spindles." PLoS One **2**(4): e341.
Rampin, C., R. Cespuglio, et al. (1991). "Immobilisation stress induces a paradoxical sleep rebound in rat." Neurosci Lett **126**(2): 113-118.
Rauchs, G., B. Desgranges, et al. (2005). "The relationships between memory systems and sleep stages." J Sleep Res **14**(2): 123-140.
Ravassard, P., B. Pachoud, et al. (2009). "Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus." Sleep **32**(2): 227-240.
Rechtschaffen, A., B. M. Bergmann, et al. (1999). "Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat." Sleep **22**(1): 11-31.
Romcy-Pereira, R. and C. Pavlides (2004). "Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP." Eur J Neurosci **20**(12): 3453-3462.
Ruskin, D. N., K. E. Dunn, et al. (2006). "Eliminating the adrenal stress response does not affect sleep deprivation-induced acquisition deficits in the water maze." Life Sci **78**(24): 2833-2838.
Smith, C. (1995). "Sleep states and memory processes." Behav Brain Res **69**(1-2): 137-145.
Smith, C. and G. M. Rose (1996). "Evidence for a paradoxical sleep window for place learning in the Morris water maze." Physiol Behav **59**(1): 93-97.
Smith, C. and G. M. Rose (1997). "Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze." Behav Neurosci **111**(6): 1197-1204.
Smith, C. and P. T. Wong (1991). "Paradoxical sleep increases predict successful learning in a complex operant task." Behav Neurosci **105**(2): 282-288.

Smith, C. T., J. M. Conway, et al. (1998). "Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task." Neurobiol Learn Mem **69**(2): 211-217.
Stickgold, R. and M. P. Walker (2005). "Sleep and memory: the ongoing debate." Sleep **28**(10): 1225-1227.
Suchecki, D., B. Duarte Palma, et al. (2000). "Sleep rebound in animals deprived of paradoxical sleep by the modified multiple platform method." Brain Res **875**(1-2): 14-22.
Suchecki, D., L. L. Lobo, et al. (1998). "Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation." J Sleep Res **7**(4): 276-281.
Suchecki, D., P. A. Tiba, et al. (2002). "Hormonal and behavioural responses of paradoxical sleep-deprived rats to the elevated plus maze." J Neuroendocrinol **14**(7): 549-554.
Suchecki, D. and S. Tufik (2000). "Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat." Physiol Behav **68**(3): 309-316.
van Hulzen, Z. J. and A. M. Coenen (1981). "Paradoxical sleep deprivation and locomotor activity in rats." Physiol Behav **27**(4): 741-744.
Vertes, R. P. (2004). "Memory consolidation in sleep; dream or reality." Neuron **44**(1): 135-148.
Vertes, R. P. and J. M. Siegel (2005). "Time for the sleep community to take a critical look at the purported role of sleep in memory processing." Sleep **28**(10): 1228-1229; discussion 1230-1223.
Wang, G. P., L. Q. Huang, et al. (2009). "Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation." Neuroreport **20**(13): 1172-1176.
Youngblood, B. D., J. Zhou, et al. (1997). "Sleep deprivation by the "flower pot" technique and spatial reference memory." Physiol Behav **61**(2): 249-256.

Chapter 4

REM sleep and Learning Following 4 Training Trials Per Day in the Morris Water Maze

Abstract

Though much of the literature indicates performance deficits in spatial learning when rapid eye movement (REM) sleep restriction immediately follows training, prior work in our laboratory using 12 training trials per day in the Morris water maze did not result in concurrent performance deficits, only later reversal learning deficits. As it was possible the lack of deficit was a result of overlearning prior to the REM sleep deprivation period, I investigated the effect of REM sleep restriction following training using only 4 trials per day in the Morris water maze. Rats were divided into 3 groups: controls (CON, n = 9), REM sleep restricted during the initial spatial learning phase (rRSL, n = 8) and REM sleep restricted during the reversal phase (rRSR, n = 9). All groups performed 4 training trials per day for 6 days, with an additional probe trial at the start of Days 4 and 6. From

the 7th trial on the 4th day, rats were trained on a reversal learning phase of the task. When not being REM sleep deprived for 6 hrs immediately following training, rats were returned to their homecages. I found that rRSL as compared to CON did show performance deficits on the concurrent initial spatial learning phase, and instead of deficits, had performance enhancements during the subsequent reversal learning phase. In contrast, rRSR had no performance differences on the concurrent reversal learning phase as compared to CON. The results suggest that reversal learning is protected from the effects of concurrent REM sleep restriction. In comparison with my previous findings, the effects of REM sleep restriction on concurrent initial spatial learning and subsequent reversal learning are dependent on the number of training trials per day in the Morris water maze.

Introduction

Over the years, there has been much debate over the impact of REM sleep deprivation (RD) on learning (McGrath and Cohen 1978; Smith, 1985, 1995; Morrison, Sanford et al. 2000; Stickgold and Walker 2005; Vertes & Eastman, 2000; Vertes and Siegel 2005). Smith (1985) proposed that the null effect could result from sufficient learning of the task prior to RD or too simple a task to require memory processes during RS. Prior to Smith's (1985) review, the preponderance of research on the interaction of RS and learning used avoidance tasks, with only a few focusing on spatial learning and none using the Morris

water maze (Morris 1984). Since then, the interaction of RS and spatial learning has been more thoroughly investigated.

A number of the more recent studies report that RD results in performance deficits in spatial learning following both long periods of RD (24 – 72 hrs: Youngblood, Zhou et al. 1997; Ruskin, Dunn et al. 2006; Li, Tian et al. 2009; Wang, Huang et al. 2009) and short periods of RD (4 – 6 hrs: Smith and Rose 1996; Smith and Rose 1997; Smith, Conway et al. 1998; Bjorness, Riley et al. 2005). I showed in Chapters 2 and 3 that RS restriction (rRS, short bouts of RD) did not affect next day performance when administered during initial spatial learning or during reversal learning when the hidden platform was moved to the opposite side of the Morris water maze tank while maintaining all distal cues in their original positions. However, I did see that rRS during initial spatial learning produced a subsequent deficit in performance during reversal learning (Chapter 2).

To date, there is a large amount of non-performance related studies indicating a link between REM sleep (RS) and measures of synaptic plasticity, the presumed building block of learning. For example, an increase in zif-268, an immediate early gene marking synaptic plasticity, was increased to a level similar to that of active learning during the first few RS bouts following exposure to a novel environment and following long-term potentiation (LTP) induction (Ribeiro, Goyal et al. 1999; Ribeiro, Mello et al. 2002). Studies measuring LTP in the

hippocampus have found RD related deficits on subsequent LTP (Davis, Harding et al. 2003; McDermott, LaHoste et al. 2003; Romcy-Pereira and Pavlides 2004; Kim, Mahmoud et al. 2005; Ravassard, Pachoud et al. 2009). When RD was administered immediately after LTP induction, impairments in LTP maintenance were found even after only 4 hrs of RD (Ishikawa, Kanayama et al. 2006).

During both active waking and RS, a sinusoidal rhythm in the theta band frequency (4 - 10 Hz) is present in the hippocampus. Further evidence for an interaction of RS and learning came when it was shown that during maze running across 4 days, hippocampal cells associated with a novel maze fired at theta peaks while running the maze and during RS replay. By the 4^{th} day when the task was well learned, the hippocampal cells associated with the task reversed the phase of firing to theta troughs during RS (Poe, Nitz et al. 2000). Firing at theta troughs during RS replay was specific to hippocampal cells associated with either a familiar maze or after four days of running a novel maze. Hippocampal cell firing during RS also has been shown to have the same timescale of replay as when running the task (Louie and Wilson 2001).

It is thought that hippocampal-dependent learning and consolidation occurs via the interplay between synaptic potentiation and depotentiation. Hölscher et al. (1997) described that in vitro stimulation during theta peaks induced LTP, and stimulation during theta troughs led to considerable depotentiation of already potentiated synapses. Depotentiation is blocked with the presence of serotonin

(Kemp and Manahan-Vaughan 2004; Kemp and Manahan-Vaughan 2005) and norepinephrine (NE) (Katsuki, Izumi et al. 1997; Yang, Lin et al. 2002), both of which are distinctly reduced during RS (Iwakiri, Matsuyama et al. 1993; Portas and McCarley 1994; Park, Lopez-Rodriguez et al. 1999; Shouse, Staba et al. 2000; Penalva, Lancel et al. 2003). In a study of NE depletion and reversal learning, it was shown that the lack of NE was associated with enhanced reversal learning in the 8-arm maze (Harrell, Barlow et al. 1984), suggesting that RS may facilitate reversal learning.

Based on the evidence presented in other studies (e.g. Smith and Rose 1997), I had expected rRS during initial spatial learning would result in a deficit in performance using 12 training trials per day (12TpD) in the Morris water maze. I had previously hypothesized that rRS during reversal learning would also result in a deficit in performance as reversal learning has been described as being more susceptible to changes in hippocampal activation (Pouzet, Welzl et al. 1999; Cirulli, Berry et al. 2000; Cirulli, Berry et al. 2004). However, I found that rRS following 12 daily trials of training did not result in a deficit in performance during either concurrent initial spatial learning or concurrent reversal learning (Chapter 2). In an attempt to determine if the lack of a rRS-associated performance deficit was a result of the RD technique, I altered an aspect of our deprivation technique to mimic that used by others (high water in the RD chambers). However, I still did not produce a rRS-associated performance deficit

during initial spatial learning or concurrent reversal learning in comparison to my controls (Chapter 3).

As the null results for the effects of rRS on initial spatial learning were consistent across my two 12TpD studies (Chapters 2 and 3), I postulated that the rats may have sufficiently learned the task within the first day of training, prior to any RS manipulations, i.e. that the 12TpD were sufficient for complete learning and would not require memory processes during RS. It was possible that the lack of deficit with rRS I observed resulted from a plateau effect, as suggested by Smith (1985). Therefore, I wanted to repeat my earlier experiment (Chapter 2) to investigate if rRS administered following fewer trials per day in the Morris water maze would affect either initial spatial learning or reversal learning.

Smith and Rose (1997) used 12 training trials in the Morris water maze prior to rRS just as I did (Chapters 2 and 3), but unlike my results they saw significant differences in learning from day 1 to 2. However, the level of enriched distal cues within their testing environment may have been considerably lower than mine. Twelve trials in a room with several cues, as in my study, may result in complete learning within the first day of training as compared to 12 trials with few cues. In my previous study (Chapter 2), performance measures at the end of day 1 did not differ from those at the end of day 2, which suggests learning was near saturation prior to the first rRS session. Incomplete learning during the first day may leave the consolidation process more vulnerable to the effects of rRS.

The goal of my current study was to follow up on Smith's earlier proposal (1985) that RD may have a greater effect on performance if administered after insufficient on-line learning has occurred. I use fewer trials, but an otherwise identical paradigm and room environment to my previous work (Chapter 2) to isolate if fewer trials would lead to a rRS-associated performance deficit during initial spatial learning and / or reversal learning. Based on previous evidence for the link between RS and learning, I predicted that my prior findings of rRS not affecting either initial spatial learning or reversal learning was an effect of knowing the task too well. Therefore, I would expect to see a performance deficit when learning was not saturated by the end of each training day in validation of Smith's memory saturation proposal. When looking at Day 1's trial-by-trial performance in the 12TpD studies (Chapters 2 and 3), I found that learning had not saturated by trials 4 to 6, therefore I tested the effects of RD on learning using 4 training trials per day (4TpD). I hypothesize that RD immediately following training with 4TpD for initial spatial learning or reversal learning would cause a deficit in performance. Based on the theory that RS facilitates learning, I further hypothesized that no deficit in learning would occur after release from rRS for subsequent reversal learning. Moreover, I could see an improvement in subsequent reversal learning performance in the previously rRS rats as compared to controls, if during initial spatial learning the rRS group relied on hippocampus-independent strategies, which could facilitate the efficiency in learning the new platform location.

Methods

Rats were tested for the effect of REM sleep deprivation on initial spatial learning, concurrent reversal learning and subsequent reversal learning when 6 hrs of RD was administered immediately after 4TpD in the Morris water maze.

Animals

All rats used in this study were Sprague-Dawley male rats (~390 g; Harlan Indianapolis, IN). Animals were housed in a 12:12 light cycle at an average temperature of 23 °C. Procedures were approved by the animal review board, the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. Rats had *ad libitum* access to fresh drinking water and food at all times except while in the water maze. As stress can impair learning (Foy, Stanton et al. 1987) and can be a side-effect of RS deprivation, I chose to use changes in percent body weight as my measure of stress levels (e.g. van Hulzen and Coenen 1981). Each rat was weighed at the start of each experimental day, before testing, to monitor changes in percent body weight.

REM sleep deprivation protocol

I administered the identical REM sleep deprivation protocol as I used in studying the effects of low level water RD on learning with 1212TpD (Chapter 2). Rats

were RS deprived using 3 inverted flowerpots and a low level of water (2 cm in height) in the deprivation chambers.

Visual Water Maze protocol

The task used to test the rats' ability to locate the visible platform was the same as previously described in Chapter 2.

Morris Water Maze protocol

Rats were randomly assigned into one of 3 groups: Controls (CON; n = 9), delayed RS restricted during the learning phase (rRSL; n = 8) and RS restricted during the reversal phase (rRSR; n = 9). Aside from the number of training trials per day, the Morris water maze protocol was identical to that used in both Chapters 2 and 3. It was a 6 day protocol. The Learning Phase consisted of the initial spatial learning component of the study, lasting for the first 3 and a half days of the study with the platform located in the Northeast quadrant. For the Reversal Phase, the platform location was changed to the opposite side of the tank, while maintaining all room cues in their current position. The Reversal Phase started on the 3rd trial on Day 4 and persisted for the remainder of the experiment. Learning was tested using a probe trial at the start of Day 4 and Day 6 (see Figure 4.1 for the protocol outline). In total 4 training trials were run each day. Four trials were chosen as a suitable number of training trials per day, based on the performance observed in the control group in my previous work

(Chapters 2 and 3). I measured latency to platform, pathlength, velocity and the Gallagher cumulative distance from platform during the training trials. The indices of learning I measured on probe trials were: number of platform crossings, Gallagher's average proximity to the platform and percent time spent in the target quadrant. In addition, velocity and pathlength were also measured for the probe trials.

RD was for 6 hrs, starting immediately after the 10 min drying period following training in the Morris water maze. The rRSL group was RS deprived for the first 6 hrs immediately following training on Days 1, 2 and 3 during the Learning Phase. The rRSR group was RS deprived for 6 hrs immediately following training on Days 4 and 5 during the Reversal Phase.

Statistics and data analyses

Data were analyzed as trialsets (average performance across 2 consecutive trials: Trials 1 and 2; Trials 3 and 4) and in specific cases as single trials. Retention was measured by comparing the last trial of a day with the first trial of the subsequent day. Retention was also calculated for trialset 1 (trials 1 and 2) versus trialset 2 (trials 3 and 4) the following day.

To attain a measure of initial differences in swim preference (how close the rat swam to the target platform) I analyzed the first 5 s of the trials for the cumulative distance measure. The first 5 s of the trial length was chosen for analysis as we

thought it would allow sufficient time for the rat to orientate to the Morris water maze and start along its swim path. Similarly, for the probe trials I analyzed both the entire trial length and the first 10 s alone to determine if there were initial differences between groups that may later be washed out when analyzing the entire trial length. I allowed a longer initial window for the probe trial (first 10 s) as compared to training trials as the removal of the platform for the probe trial protected against the rat finding the platform without knowledge of its location. This initial window could indicate initial preference while a longer or later time window may indicate that rat's persistence in searching within the same location rather than searching elsewhere when the platform was not located.

For rRSR, performance between groups was similar at the start of the Reversal Phase. Therefore, no performance measures were normalized for this study.

Statistical analyses were similar to those used in Chapter 2. The Learning Phase was analyzed with 2 trialsets per day for 3 days (Days 1, 2 and 3). To determine differences within each day, RMANOVA were used across the 2 trialsets on Days 1, 2 and 3, and across the first trialset on Day 4. Retention at the start of Days 2 and 3 was analyzed using a one-way ANOVA on the difference between the last trialset on Day 2 and the first trialset on Day 1. Similarly retention was assessed for the start of Day 3 as the difference between the last trialset on Day 2 and the first trialset on Day 3. Retention was also assessed using individual trials. The Reversal Phase was analyzed with 2 trialsets per day for 2 days (Days 5 and 6).

To determine differences within each day, RMANOVA were used across the 2 trialsets on Days 5 and 6, and across the last trialset on Day 4. Retention at the start of Day 5 was analyzed using a one-way ANOVA, similar to the Learning Phase. This analysis was also done for a single trial instead of the trialset.

In order to determine how the difference in training load affected the performance results I statistically compared performance measures for the control groups from both this current study with previous work in our lab using 12 trials (Chapter 2). In addition, I also compared performance for the RS deprived during learning groups from both studies, for the initial Learning Phase and the subsequent Reversal Phase. I used independent *t*-tests comparing the performance measure for each day between the average of the current 4TpD study to the average of the first 4 trials (trials 1 - 4), middle 4 trials (trials 5 – 8), and last 4 trials (trials 9 – 12) of the previous 12TpD study. To compare performance between the two control groups on Day 4, the first 2 trials alone on Day 4 were used for the Learning Phase, as were the first 2 trials alone of reversal training on Day 4 used for the Reversal Phase. I used similar comparisons when comparing the differences in performance between groups rRS during the initial Learning Phase for the 12TpD and 4TpD studies.

To determine whether performance during the Learning Phase was predictive of Reversal Phase performance, I ran a series of correlations. I correlated performance on the Day 4 probe trial with performance in relation to the Reversal

Phase platform location on the Day 6 probe trial. I also correlated performance on the average of the first 2 trials for the first 5 s of the cumulative distance measure between the start of Day 2, following the first rRS session during the Learning Phase with performance at the start of Day 5, following the first night of homecage sleeping during the Reversal Phase. For the correlations, I collapsed across both studies' control groups (4TpD controls and 12TpD controls) and rRSL groups (4TpD rRSL and 12TpD rRSL) to isolate the effect of rRS on between-phase correlations. As the number of subjects was relatively low for within group correlations (controls 12T: n = 7; rRSL 12T: n = 10; controls 4T: n = 9; rRSL 4T: n = 8) I retained all of the rats or data samples, although in some cases there were deviations from the main group, which could have driven the trendlines. The subject number was too low within each individual group to ascertain an accurate representation if an interactive effect was present between rRS and training load.

Results

The goal of this study was to determine how rRS, immediately following training, affected performance with 4 training trials per day in the Morris water maze during both initial spatial learning and reversal learning.

The effects of rRS during the Learning Phase

During the Learning Phase probe trial on Day 4, when the first 10 s of the probe trial were analyzed, rRSL had significantly less preference for the learning phase platform location than CON (Percent time in target quadrant: $p = 0.048$, Figure 4.2; Gallagher's average proximity to platform: $p = 0.036$, Figure 4.3). When the entire 60 s trial was analyzed, all rats showed a strong preference for the Learning Phase platform location though no group differences were found with any of the variables measured (percent time in target quadrant, Figure 4.4; average proximity to platform, Figure 4.5), including velocity. The differences identified during the probe trial indicate that initially, CON searched the target area more than rRSL, therefore showing a better level of initial retention than rRSL. This indicates that 6 hrs of RD during initial spatial learning with 4TpD disrupted learning.

This deficit associated with RD during the Learning Phase was further evidenced during training. While all groups improved across the Learning Phase (latency to platform, pathlength and the entire trial length for cumulative distance), when the first 5 s of the trials were analyzed for differences in cumulative distance from the platform, rRSL was impaired on Day 2 ($p = 0.017$) as compared to CON (Figure 4.6). This difference seemed to be driven by a large deficit for rRSL in performance on the second trialset (trials 3 and 4) as compared to CON ($p = 0.017$). By Day 3, performance between the two groups was more equivalent. Interestingly, in a paired t-test (as used in Smith and Rose, 1997) for Day 1

versus Day 2, CON significantly improved their latency performance on Day 2 as compared to Day 1 ($p = 0.009$, Figure 4.7). In contrast, rRSL did not improve their latency performance on Day 2 as compared to Day 1. This suggests that RD during initial spatial learning delays or hinders performance improvements.

When retention was analyzed for the differences between performance at the end of training and the start of training the subsequent day, no group differences were identified for latency, pathlength or cumulative distance. When the first 5 s of the trials were analyzed for retention, a group difference was identified where there was a significant improvement in retention for rRSL compared to CON between the end of Day 2 and the start of Day 3. When both performance at the start of Day 3 and the performance plot across the Learning Phase were considered (Figure 4.6), it was clear that CON were not impaired, and that this improvement in retention for rRSL was driven by the significantly poorer performance at the end of Day 2 for rRSL. The parity in performance between the two groups at the start of Day 3 indicates that rRSL were not performing better than CON.

rRSL and CON swam at similar speeds throughout the Learning Phase.

Summary of the effects of rRS during the Learning Phase

RD during initial spatial learning of 4TpD results in a deficit in consolidation following RD as measured on Day 2 and on the initial heading on the Day 4 probe trial (Table 4.1A).

The effects of rRS during the Reversal Phase

During the Reversal Phase probe trial on Day 6, when either 60 s or the first 10 s of the probe trial was analyzed, no significant differences were found between rRSR and CON in any of the variables measured (percent time in Reversal Phase quadrant, percent time in Learning Phase quadrant, number of Reversal Phase platform location crossings, number of Learning Phase platform location crossings, average proximity to Reversal Phase platform location, average proximity to Learning Phase platform location, pathlength or velocity).

In a series of paired t-test comparisons to test for differences in preference within each group for one platform location over the other during either the entire 60 s or first 10 s of the probe trial, neither CON nor rRSR had a stronger preference for the Learning Phase platform location over the Reversal Phase platform location (Figures 4.8 and 4.9). Performance was compared for the Learning Phase platform location on Day 4 versus the Reversal Phase platform location on Day 6, and both CON and rRSR did better on Day 4 than on Day 6 for percent time in target quadrant ($p = 0.008$, Figure 4.8) and average proximity to platform ($p = 0.027$, Figure 4.9) for the 60 s trial. When only the first 10 s of the probe trial

were analyzed, while CON had a trend for swimming closer to the target platform location on Day 4 ($p = 0.057$), rRSR did significantly better on Day 4 than on Day 6 for percent time in target quadrant ($p = 0.008$) and average proximity to target platform ($p = 0.027$). Interpretations of the paired t-test results could suggest that during the initial part of the probe trials, rRS may have slightly impaired Day 6 performance due to the significantly better performance on Day 4 for rRSR and only a trend for better performance for CON.

During reversal training, both groups improved (latency to platform, pathlength and cumulative distance to platform) but no group differences were identified, and both rRSR and CON had similar swim speeds.

Summary of the effects of concurrent rRS on Reversal Learning

RD during reversal training with 4TpD overall does not affect reversal learning (Table 4.2).

The effects of prior rRS concurrent with initial spatial learning on subsequent reversal learning

When rRS during the Learning Phase was discontinued for the Reversal Phase, the probe trial on Day 6 revealed that the rRSL group significantly preferred the Reversal Phase platform location compared to CON (Average proximity to Reversal Phase platform location: $p = 0.03$, Figure 4.3) when the entire 60 s trial

was analyzed. Further, trends for group differences identified that rRSL spent more time in the Reversal Phase target quadrant ($p = 0.084$, Figure 4.2) than CON, while CON spent more time in the Learning Phase target quadrant ($p = 0.075$) and swam in closer proximity to the Learning Phase platform location ($p = 0.051$) than rRSL. Paired t-tests indicated that on Day 6, rRSL had a strong preference for the Reversal Phase platform location over the Learning Phase platform location (percent time in target quadrant: $p = 0.001$; average proximity to platform: $p < 0.001$). CON, however, had no such preference, showing a similar preference level for both platform locations. When the target platform locations were compared between the Learning Phase platform location on Day 4 with the Reversal Phase platform location on Day 6, CON performed better on Day 4 as compared to Day 6 (percent time in target quadrant: $p = 0.04$; average proximity to platform: $p = 0.044$). rRSL had similar performance levels for the target platform location on Day 4 (initial platform) and Day 6 (reversal platform).

When only the first 10 s of the Reversal Phase probe were analyzed, CON also tended to spend more time in the Learning Phase target quadrant than rRSL ($p = 0.079$). When paired t-tests were analyzed for differences within group between the two platform locations on the Day 6 probe trial, rRSL again had a stronger preference for the Reversal Phase platform location (average proximity to platform: $p < 0.001$, Figure 4.3; percent time in target quadrant: $p = 0.002$, Figure 4.2), while CON did not. Further in a comparison of the first 10 s between the two probe trials, CON tended to swim closer to the Learning Phase platform location

on Day 4 than the Reversal Phase platform location on Day 6 ($p = 0.057$). Overall, the results from the analyses on the probe trial suggest that rRS during initial spatial learning with 4TpD facilitates subsequent reversal learning, just as it impairs learning of the initial target platform location compared to normal sleeping controls.

On the first trial of reversal learning, rRSL tended to take longer to find the platform than CON ($p = 0.089$). No other variables identified this group difference, and performance was equivalent between the two groups on the second reversal trial. Otherwise, no group differences were identified for any of the training variables (latency, pathlength, cumulative distance from the platform) when either the entire trials or the first 5 s of the trials were analyzed. Additionally, no differences were identified in the level of retention between the two groups at the start of Day 5 as compared to the end of Day 4. However, in a paired t-test comparing latency performance between the reversal trialset on Day 4 and the average performance on Day 5, rRSL significantly improved on Day 5 as compared to Day 4 ($p = 0.009$), while CON did not (Figure 4.6).

Summary of the effects of rRS on subsequent reversal learning

rRS during initial spatial learning of 4 trials per day appears to lead to a facilitation of subsequent reversal learning (Table 4.1 B).

Body weight

rRSL and CON body weights were compared as a percent of Day 1 body weight. When each day was analyzed, no group differences were found (Figure 4.10). In an analysis across the Learning Phase, no group differences or interactions were identified. Across the Reversal Phase, there was a trend for a day x group interaction ($p = 0.097$) where rRSL had less percent body weight on Day 5 than Day 6, while CON were more stable. rRSR and CON body weights were compared as a percent of Day 4 body weight. When each day was analyzed, a trend for rRSR to have greater percent body weight loss compared to CON on Day 6 was identified ($p = 0.057$, Figure 4.11). On Day 5, percent body weights were equivalent between groups. Across the Reversal Phase, a day x group interaction was found ($p = 0.018$) which was driven by the difference in percent body weights on Day 6.

Summary of the rRS effect on percent body weight

Overall my data indicate that rRS was not associated with significant changes in percent body weight.

Comparison of results for control groups from the 4 training trial per day study and 12 training trial per day study

For performance at the end of Day 1, the 12TpD control group was significantly better than the 4TpD control group ($p \leq 0.001$), though both groups were

equivalent at the start of Day 1 (latency, pathlength and cumulative distance measures). At the start of the Reversal Phase, the 12TpD control group performed significantly better than the 4TpD control group on the cumulative distance measure ($p = 0.023$), though performance was equivalent for both latency and pathlength measures. On all other training comparisons, performance across the Learning Phase and Reversal Phase was better for the controls that received 12TpD for latency, pathlengh and cumulative distance as compared to the controls that received only 4TpD.

Control group comparisons across probe trials

As expected during the probe trial on Day 4, the control rats that received 12TpD performed significantly better than the 4TpD controls for pathlength ($p = 0.049$), percent time spent in the Learning Phase target quadrant ($p = 0.003$), the number of platform crossings ($p = 0.013$) and the average proximity to the Learning Phase platform ($p < 0.001$).

Unexpectedly, on the Day 6 probe trial, performance between the two control groups was equivalent on pathlength, percent time spent in both the Reversal Phase and Learning Phase target quadrants, number of Learning Phase platform crossings, average proximity to both the Learning Phase and Reversal Phase platforms. However, the 12TpD control group was significantly better than the 4TpD control group for the number of Reversal Phase platform crossings.

Summary of performance for control groups in the 4 training trial per day and 12 training trial per day studies

At the start of the experiment, performance was equivalent between the 4TpD and 12TpD control groups However the additional 8 training trials per day strongly benefited performance across both the Learning Phase and the Reversal Phase, the 12TpD control group performed better than the 4TpD control group. Interestingly, performance was fairly equivalent for both control groups on the Day 6 probe trial.

Comparisons across the training trials for the rRS during initial Learning Phase groups in the 12 training trial per day and 4 training trial per day studies

I compared performance between the rRS during Learning Phase groups from the previous 12TpD study and the current 4TpD study. As expected, performance on the 12TpD study was significantly better than the 4TpD study ($p < 0.05$, latency and cumulative distance to platform) and both groups were equivalent for the first 4 trials on Day 1. On the pathlength measure, the 12TpD rRSL group performed significantly better than the 4TpD rRSL group starting midway through the second day ($p = 0.011$) continuing throughout the Learning Phase. At the start of Day 4 following the probe trial, latency measures were again equivalent between both rRS groups, suggesting that the probe trial re-equilibrated performance between the 12TpD rRSL and 4TpD rRSL groups. As expected, on all measures performance was also equivalent at the start of the

Reversal Phase on Day 4 and equivalent again immediately following the probe trial on Day 6. All other analyses for training during the Reversal Phase indicated that the 12TpD rRSL group performed significantly better than the 4TpD rRSL group.

Comparisons across the probe trials for the rRS during initial Learning Phase groups in the 12 training trial per day and 4 training trial per day studies

During the probe trial on Day 4, pathlength was equivalent between the two groups, but similar to the comparison of the control groups between the two studies, for percent time spent in target quadrant ($p < 0.001$), number of platform crossings ($p = 0.006$) and average proximity to platform ($p < 0.001$) the 12TpD rRSL group performed significantly better than the 4TpD rRSL group.

Unlike the comparison of the 12TpD and 4TpD control groups on the Day 6 probe trial, the 12TpD rRSL group showed a stronger preference for the Learning Phase platform location as compared to the 4TpD rRSL group as measured by percent time spent in Learning Phase target quadrant ($p < 0.001$), number of Learning Phase platform crossings ($p = 0.011$) and average proximity to Learning Phase platform location ($p < 0.001$). The 12TpD rRSL group had significantly more Reversal Phase platform crossings than the 4TpD rRSL group ($p = 0.025$), but the 4TpD rRSL group performed better for the Reversal Phase platform location than the 12TpD rRSL group as measured by percent time spent in

Reversal Phase target quadrant ($p = 0.017$) and average proximity to the Reversal Phase platform ($p = 0.001$). Similar to controls, both rRSL groups were equivalent for pathlength.

Summary of performance for rRSL groups in the 4 training trial per day and 12 training trial per day studies

While performance was equivalent between both rRSL groups at the start of the experiment, those that received more trials benefited across training on both the Learning Phase and the Reversal Phase. However, on the probe trials, while the rRSL group that had 12TpD clearly knew the Learning Phase platform location better on Day 4 than those that received only 4TpD, those that received fewer training trials had better retention of the Reversal Phase platform area, though not the precise location.

Analyses of the relationship between Learning Phase and Reversal Phase performance

Cumulative distance performance for the first 5 s of the trial following the first night with either rRS or homecage sleeping (first 2 trials at the start of Day 2) during the Learning Phase was compared to performance following the first homecage night following reversal training (first 2 trials at the start of Day 5). Learning Phase performance positively correlated with Reversal Phase performance for controls (12TpD and 4TpD combined) only ($r^2 = 0.481$, $p =$

0.003; Figure 4.12), indicating that poorer performers during the Learning Phase, remained the poorer performers during the Reversal Phase, though rRS disrupted this relationship ($r^2 = 0.095$). When I investigated if a relationship existed for performance during the two probe trials, I tested for significant correlations between the Learning Phase platform location on the Day 4 probe trial and the Reversal Phase platform location on the Day 6 probe trial. Controls (12TpD and 4TpD combined) had a positive relationship in between day probe performance for percent time spent in target quadrant ($r^2 = 0.253$, $p = 0.049$, Figure 4.13), number of platform crossings ($r^2 = 0.706$, $p < 0.001$, Figure 4.14) and average proximity to target platform ($r^2 = 0.339$, $p = 0.019$, Figure 4.15). In contrast, the rRSL group (12TpD and 4TpD combined) had negative correlations for both percent time spent in target quadrant ($r^2 = 0.249$, $p = 0.035$), and average proximity to target platform ($r^2 = 0.382$, $p = 0.006$). No correlation was found for rRSL on the number of platform crossings measure ($r^2 = 0.119$). This indicates that for homecage controls, poorer performers remain poor throughout the study (training and probe trials), though rRS disrupts this relationship by either removing any relationship, or by creating the opposite, where poorer performance on the Learning Phase relates to better performance on the Reversal Phase and vice versa. The numbers of subjects tested within each individual group (controls 12TpD, controls 4TpD, rRSL 12TpD and rRSL 4TpD) are too few to accurately be able to discern if the number of training trials per day interacts with rRS to further disrupt how predictive early performance is of later performance.

Summary the relationship between Learning Phase and Reversal Phase performance

It appears that rRS alters the relationship between performance during the Learning Phase and performance during the Reversal Phase. When RS is not manipulated, poorer performers are consistent across both phases. When rRS is administered, when correlated, poorer performer in one phase is associated with better performance in the other phase.

Discussion

My study indicated that when RD was administered immediately after 4 training trials of initial spatial learning, performance was impaired on the Learning Phase of the Morris water maze (Table 4.1 A). Specifically, rRS resulted in a deficit in percent time spent in the target quadrant for the first 10 s of the probe trial as compared to CON. Further, during the first 5 s of the training trials, rRS resulted in increased cumulative distance from the platform on Day 2, in particular during the latter half of Day 2, indicative of learning impairments. While latency to platform improved for CON between Days 1 and 2, rRSL did not show this improvement. Interestingly, rRS during initial spatial learning also appears to enhance subsequent reversal learning (Table 4.1 B and Figures 4.4 and 4.5). This reversal learning enhancement was seen when the rRSL group swam closer to the Reversal Phase platform location on the Day 6 probe trial, and CON

showed no preference for either platform location. Additionally, latency was reduced (improved performance) between Day 4 and Day 5 reversal training for rRSL but not for CON. When RD was administered immediately after 4 trials of reversal learning, performance was not affected (Table 4.2, Figures 4.8 and 4.9). My data and potential reasons for my findings are summarized in Figure 4.17.

To date, this is the first study to investigate the effects of rRS during 4 trials of reversal learning in the Morris water maze. I was also able to describe the differential effects of rRS on initial spatial learning and subsequent reversal learning when 4 trials as opposed to 12 trials are used during training.

The effects of rRS during initial spatial learning

When rRS was administered during the Learning Phase following 12TpD, performance was not affected (Chapter 2). However, when only 4TpD, rRS was associated with a performance deficit both during training and on the probe trial. Results from my current study are similar to what has been reported previously on the effects of rRS following training in the Morris water maze (Smith and Rose 1996; Smith and Rose 1997; Youngblood, Zhou et al. 1997; Li, Tian et al. 2009; Wang, Huang et al. 2009).

Prior work by Smith and Rose (and 1996; 1997) looked at rRS effects on both 4TpD and 12TpD during initial spatial learning in the Morris water maze. They found a rRS-associated prolonging of trial length on Day 2. I had previously

hypothesized (Chapters 2 and 3) that the lack of impairment in my 12TpD study rRS rats compared with those described by Smith and Rose (1997), arose from differences in RD technique used. However, when further research in our lab was done to address the difference in the level of water within the deprivation chamber as the key experimental RD technique difference (Chapter 3) I merely replicated our prior null results. I did not see prolonged latencies to platform associated with rRS, but only differences predominantly using the more sensitive Gallagher measures (Gallagher, Burwell et al. 1993; discussed in Hodges 1996; Maei, Zaslavsky et al. 2009). It is possible that the differences between our lab's work and that of Smith and Rose (and 1996; 1997) are a result of other aspects of the RD technique, for example, how enriched the Morris water maze testing room is with cues, or are a result of the learning ability or visual acuity of the rats used in the studies. A discussion of the potential differences between the RD techniques is included in Chapter 3. While both labs used Sprague-Dawley rats and tested the rats with the visual platform version of the Morris water maze, my rats were supplied from Harlan (Indianapolis, IN), while those of Smith and Rose were bred within the lab. Additionally, rats in our lab are pretested on the visual platform version of the water maze, while those in the Smith and Rose studies are tested after the hidden platform version of the Morris water maze. Pretraining introduces the animals to the procedural aspects of the task, which can aid them in finding the hidden platform location faster (see Hoh, Beiko et al. 1999). A training advantage at the onset could also lead to my animals sufficiently learning the task on the first day of 12 training trials in comparison to Smith and Rose's

study (1997). My current results for the rRS-associated deficit with fewer training trials could support this.

Although not the best measure of learning (Gallagher, Burwell et al. 1993; discussed in Hodges 1996), most studies report on latency to platform. Of the previous studies that investigated the effects of RD on performance in the Morris water maze reporting latency, Smith and Rose (1996), Li et al. (2009) and Wang et al. (2009) used 4 trials of training per day, and both Smith and Rose (1997) and our lab (Chapters 2 and 3) used 12 trials of training per day. Only the last 4 training trials were represented from the first day of 12 training trials, and a total of only 4 trials were run the following day in the Smith and Rose (1997) study. Thus it is difficult to determine whether Smith and Rose's (1997) rats were performing at the same level as mine, though performance appears similar. However, if I compare the performance between the two Smith and Rose papers (4TpD study: 1996; 12 training trial study: 1997) presuming that the testing room had similar cues, the performance level on day 3 with 4 trials of training per day (Smith and Rose 1996) was similar to the performance level on the last 4 trials on Day 1 of the 12 training trial study (Smith and Rose 1997). These two groups of 4 trials correspond to absolute trial numbers 9 – 12. Thus the data support that the number of trials per day does considerably change the rate of learning, and in this case remained proportional to the number of trials run. However, in the comparison of my current study using 4TpD and my previous study using 12TpD in the Morris water maze, 4TpD controls reached a similar trial duration (latency

to platform) at the end of the third day (absolute trial numbers 9 - 12) as the 12TpD controls at the start of Day 2 (absolute trial numbers 13 – 16). That is, my 12TpD rats had longer latencies to the platform than the rats in Smith and Rose's (1997) 12 training trials on Day 1 study, which may have been the result of considerable size differences in the water maze sizes, with Smith and Rose's being smaller. As an example of how differences in experimental details could affect results, both Wang et al. (2009) and Li et al. (2009) also performed 4TpD within the Morris water maze. On Day 3, all groups had longer latencies in the Wang et al. (2009) study as compared to the Li et al. (2009) study, though this also may have been a result of Wang et al. using a larger water maze size than Li et al. used. Further, in my current 4TpD study (Figure 4.7), Day 2 latency measures were more comparable to the Li et al. (2009) study's performance on Day 3 than their Day 2 performance. There are a number of factors that could lead to such performance offsets, with one likely cause being a difference in the richness of spatial cues to navigate by, which are not well documented in any of the studies.

It is possible that in my 12TpD study (Chapter 2) based on the performance at the end of Day 1 rats were able to learn the task completely within the day. Complete learning while on-task likely reduces the level of dependence on post-training synaptic plasticity and consolidation processes, rendering the rats unsusceptible to rRS following training (Smith 1985). With only 4TpD rats had not approached asymptotic learning at the end of Day 1, and may thereby be more

dependent on post-training processing or synaptic plasticity. My results support this possibility and suggest that post-training memory processes could be especially important when the task is incompletely learned, whereas it is less important following complete on-task learning.

If RD immediately following training had impaired synaptic plasticity within the hippocampus, as previously described starting after 4hrs of RD in a study of prolonged RD (48 hrs) following high frequency stimulation to induce LTP (Ishikawa, Kanayama et al. 2006), on Day 2 I would expect the observed performance deficit, as post-training spatial learning within the hippocampus may not have sufficiently taken place during the 6 hr RD period. Impaired post-training hippocampal synaptic plasticity may have forced RS deprived rats to rely on alternative strategies to locate the platform the following day.

After hippocampal damage, Conrad and Roy (1993) described a performance deficit during initial spatial learning. Our rRS period following training acted similar to their lesion of the hippocampus during initial spatial learning. This would suggest that, for rRSL, the hippocampus may not have had an active role during the Learning Phase after the first day of training and the first rRS period. As seen with Bjorness et al. (2005), using the same RD technique as I applied, rats immediately deprived of RS for 4 hrs after training relied on non-hippocampal dependent strategies for solving the maze. It has been previously shown that the Morris water maze can be solved without the hippocampus (Hoh,

Beiko et al. 1999; Cimadevilla and Arias 2008) similar to controls. Thereby, rats' performance can still improve across training when using non-hippocampal dependent strategies, though they may not be learning to the same level as those using hippocampal dependent strategies. Therefore, it is possible that after the first day of training and rRS, rRSL are no longer relying on hippocampal dependent strategies to solve the Morris water maze. Thus rRS rats could perform the task across the Learning Phase but not to the level of accuracy as CON, as seen on both Day 2 training and Day 4 probe trial performance using my most sensitive measures for initial differences (first 5 s of cumulative distance from the platform during training, and first 10 s of average proximity to the platform location during the probe trial).

The effects of rRS during reversal learning

Using the same paradigm but a differing number of trials, in both my current 4TpD results and in my previous study 12TpD (Chapter 2) I showed that rRS during reversal learning had no effect on performance. In these studies, when RD was administered immediately following reversal training on Day 4, only 6 trials of reversal learning (for the 12TpD study) or 2 trials of reversal learning (in this 4TpD study) had been run by the rats. These findings would suggest that rRS may not have an effect at all on reversal learning in this version of the Morris water maze task, or that it may only be sensitive to rRS after 1 trial of reversal learning. Therefore, learning may be complete in reversal training after only 2 exposures to the new platform location. Guzowski et al. (2001) previously

showed that rats performed a reversal learning paradigm within 1 trial to a level comparable with a well trained group of rats. However, care must be taken when interpreting my current results. Following rRS during initial spatial learning with 12TpD, it appeared that rRS did not result in a performance deficit. However, this now appears to be dependent on the level of learning prior to the first rRS period. Therefore, while my current results suggest that when RD immediately follows reversal training in the Morris water maze, there is no affect of rRS independent of the number of trials during training, further investigation is necessary before any conclusions can be made. It may be interesting to investigate if rRS has an effect on reversal learning performance after only 1 reversal trial in an environment with fewer spatial cues, or in a generally more complex task (e.g. a place response discrimination task).

The effects of rRS during initial spatial learning on subsequent reversal learning

In studying the effects of rRS during initial spatial learning on subsequent reversal learning, 12TpD resulted in performance deficits during reversal learning (Chapter 2). In contrast, however, rRSL with 4TpD resulted in performance enhancements during reversal learning. In fact, while the greater number of training trials improved overall performance during both Learning Phase and Reversal Phase training, the improvement did not override the enhancement seen in subsequent reversal learning for the rRSL group in the current 4TpD study as compared to the rRSL group in the 12TpD study. Therefore, the impact

of rRS on subsequent reversal learning performance is dependent on the interaction of rRS and the level of training. It is unlikely that the observed enhancement is a result of a RS rebound during the Reversal Phase. The 6 hrs of RD for the three days of the Learning Phase would not be expected to summate to any homeostatic pressure to recover RS. A 6 hr period of RD should be recoverable within the day, prior to testing in the Morris water maze the following day. Using our deprivation technique and chamber description, 24 hrs of RD was recovered within the first 4 hr post-deprivation period (Mashour, Lipinski et al. in review). Further, any alterations in the sleep / waking cycle resulting from the RD for 6 hrs per day during the Learning Phase should have been similar in both the current 4TpD and my previous 12TpD study (Chapter 2), where performance deficits were observed during subsequent reversal learning rather than the performance enhancements in my current study.

After hippocampal damage, Conrad and Roy (1993) found a deficit in initial spatial learning but no performance deficits during subsequent reversal learning. Additionally, administration of brain-derived neurotrophic factor (BDNF) immediately following initial learning resulted in an enhancement during reversal learning, as seen by shortened latencies to platform (Cirulli, Berry et al. 2004), that had not been previously seen when BDNF was administered prior to initial spatial learning (Cirulli, Berry et al. 2000). These studies indicate that reversal learning can be protected or enhanced independently from initial spatial learning. My current study mirrors this with RD related deficits during initial spatial learning

and enhanced performance during subsequent reversal learning. My findings suggest that 6 hrs of RD may act as a temporary hippocampal lesion, and a return to normal sleeping patterns acts to facilitate learning.

I propose a few potential causes for the enhancement of reversal learning resulting from earlier rRS as compared to controls. The Learning Phase probe trial on Day 4 indicated that the rRSL group had not learned the platform location as well as the controls, who showed a preference for the platform location from the start of the probe trial. Not knowing the initial platform location as well may have made it easier to learn the new location during the Reversal Phase. Performance on the Learning Phase positively correlated with Reversal Phase performance for controls predicting poorer performance on the Reversal Phase for poor performers on the earlier phase. In contrast, rRSL had the opposite correlation, when any was observed, indicating that poorer performance on the Learning Phase predicted better performance on the Reversal Phase and vice versa. Thus rRS-associated impairments during the Learning Phase lead to better Reversal Phase learning, possibly as the initial phase was less well-learned. This would lead to less proactive interference (interference to new learning based on previous experience or knowledge: Underwood 1957). In my current paradigm, proactive interference would result in the predominant retention of the Learning Phase platform, to the decrement of the Reversal Phase platform location (where knowing the Learning Phase platform location interfered with learning the new Reversal Phase platform location). Though rRS

may alter the vulnerability to proactive interference, performance for controls was not affected by prior learning, but instead by overall performance capability.

Another explanation for enhanced reversal learning is if rRSL was not relying on hippocampal strategies to solve the task during initial learning, then rRSL would have refined non-hippocampal strategies that could aid hippocampal strategies in locating the new platform. Conversely, as controls may have relied more on hippocampal strategies during the Learning Phase, their non-hippocampal strategies would not be as refined to aid in locating the new platform. Though typically hippocampal and non-hippocampal strategies can interfere with each other, in this case rRSL's refined alternative strategies may facilitate them finding the new platform location more consistently across the Reversal Phase, or help them locate it until a new spatial map is created. Previous work has shown that when rats are tested for reversal learning in a new environment as compared to a new target location within the same environment, learning is slower in the same environment as opposed to a different one (McDonald, Foong et al. 2007). Therefore, if rRSL have not formed a stable contextual map of the initial Learning Phase environment, they would again be at an advantage to controls. Within the confines of my current study, it is not possible for us to address this proposal further.

In contrast to my current results, previous work done in our lab (Chapter 2), investigating the effects of rRS during spatial learning on subsequent reversal

learning with 12TpD, mirrors literature indicating that subsequent reversal learning is more sensitive to damage of the hippocampus than initial spatial learning (Pouzet, Welzl et al. 1999). This suggests that different interactions may be occurring as a result of the level of training achieved during the earlier learning and rRS manipulation period.

General Discussion

As RD is thought to impair both concurrent (Ishikawa, Kanayama et al. 2006) and future (Davis, Harding et al. 2003; McDermott, LaHoste et al. 2003; Romcy-Pereira and Pavlides 2004; Kim, Mahmoud et al. 2005; Ravassard, Pachoud et al. 2009) synaptic plasticity within the hippocampus, RS deprived rats would not be able to rely on post-training synaptic plasticity to solve the spatial task and would have to switch to less efficient alternative strategies. This change in strategy would likely result in performance deficits as compared to animals using their hippocampus to solve the spatial task. My current results support this, where performance was impaired when rRS was administered during initial spatial learning, though subsequent reversal learning was enhanced (see Figures 4.17 and 4.18).

In contrast, rats with 12TpD (Chapter 2) may be able to completely learn the task using a spatial learning strategy during training. Therefore, throughout the Learning Phase, I would not expect to see any deficits in performance with 12TpD, as the rats could retain what was learned on-line during the first day of

training. However, the RD immediately following training may still inhibit depotentiation of synapses during learning. Prior studies have suggested that RS is important for allowing depotentiation for accurate integration of new information within an old schema to occur (Poe, Nitz et al. 2000) for maximal efficiency in learning. Specifically it is thought that both the unique neurochemical milieu (Harrell, Barlow et al. 1984; Katsuki, Izumi et al. 1997; Yang, Lin et al. 2002; Kemp and Manahan-Vaughan 2004) associated with RS and the EEG sinusoidal theta rhythm in the hippocampus (Holscher, Anwyl et al. 1997) allow bidirectional synaptic plasticity for both LTP and depotentiation during learning and memory consolidation. The performance deficits in the rRSL 12TpD group during subsequent reversal learning could result from the prior Learning Phase map not being adequately depotentiated as the memories are consolidated outside the hippocampus. Without depotentiation, the Learning Phase map may remain as a dominant map, thus somewhat saturating the hippocampus with the old platform location, making it harder to learn an additional novel maze during the Reversal Phase (an example of proactive interference). Alternatively, the rRSL deficit with 12TpD during subsequent reversal learning could be an indication of impaired flexibility due to earlier rRS affecting the spatial representation of the platform location in the maze (see Figures 2.18 and 4.18).

Statistical comparisons between my current study using 4TpD and my previous work using 12TpD (Chapter 2) indicates that the number of training trials per day does impact performance. Though we were not able to determine if training load

interacts with sleep manipulations to alter the relationship between performance on the Learning and Reversal Phases, sleep manipulations alone do. Specifically, rRS performance levels (12TpD and 4TpD collapsed together) on the Learning Phase had an inverse relationship with performance levels on the Reversal Phase, overriding the differentiation in low performers versus better performers seen in the control group (12TpD and 4TpD collapsed together) across the two phases.

My findings suggest that RS is important for memory processing during initial spatial learning (Figure 4.18). Though the importance of RS cannot be detected initially when the animals have had sufficient training prior to rRS, it still impacts the memory processing, as can be seen during subsequent reversal learning. The lack of rRS effect on initial spatial learning, in my 12TpD study, could be the result of knowing the task too well prior to rRS, as suggested by Smith (1985). Though I found that rRS during reversal learning does not affect performance, in light of my results on initial spatial learning and subsequent reversal learning, this remains an open question. It would be interesting to investigate the effects of rRS following a single trial of reversal learning, or my current paradigm using varying levels of enrichment of room cues. It would also be of interest to determine the whether administering a second Reversal Phase would result in subsequent performance deficits following concurrent rRS during the first Reversal Phase.

My current study is the first study to investigate the effect of rRS on initial spatial learning, reversal learning and the later effects on subsequent reversal learning using 4TpD. Moreover, this is one of the few studies to indicate the change in rRS-associated effect on performance depending on the number of training trials in the Morris water maze, identifying an interaction between rRS and training load. Lastly, it draws attention to a potential misinterpretation of data when a manipulation initially has no effect because a hidden or latent one may exist if further investigated.

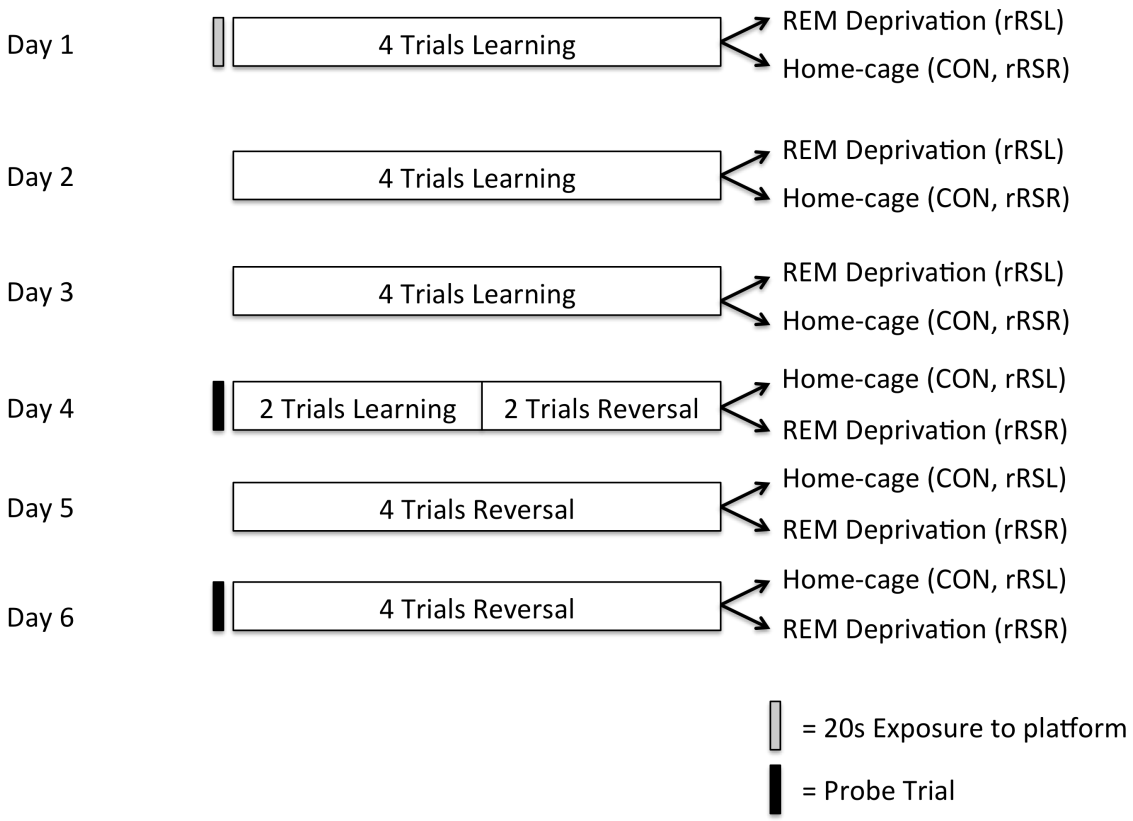


Figure 4.1 Experiment protocol
There are 6 days within the protocol. Each day had 4 trials, with an additional probe trial on Days 4 and 6. At the start of Day 1, the rats were placed on the hidden platform for 20 s. From the 3rd trial Day 4 onwards, the training trials changed from learning trials to reversal trials, when the platform was placed in the opposite quadrant as compared to the learning trials. CON and rRSR on days 1, 2 and 3 were returned to their home-cages, as were CON and rRSL on days 4 and 5. Following training on days 1, 2 and 3, REM deprivation was administered for rRSL. Following training on days 4 and 5, REM deprivation was administered for rRSR. REM deprivation was for 6 hrs immediately after the water maze. Probe trials are indicated as solid black rectangles. The initial habituation 20 s period is indicated as a solid grey rectangle.

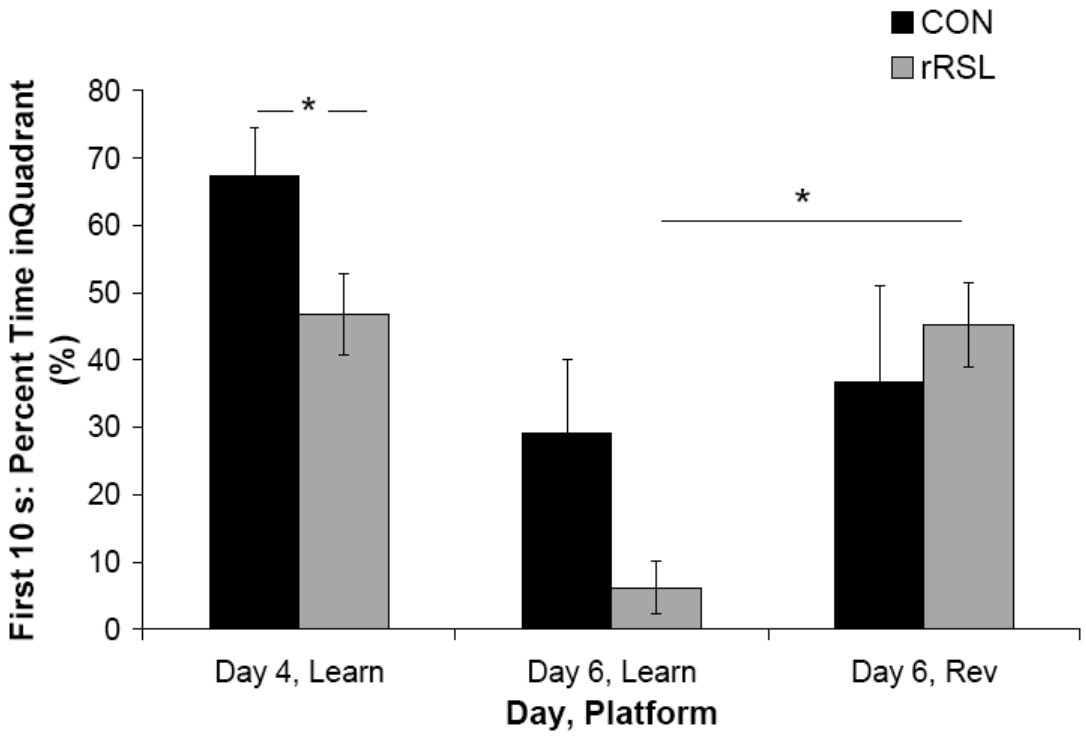


Figure 4.2 Percent time in target quadrant during the first 10 s of the Probe trial

For the percent time in target quadrant for the first 10 s of the probe trial, rRSL were significantly worse than CON on Day 4. Data are shown as mean ± SEM for CON (black) and rRSL (grey). Probe trial data for the Learning Phase target platform on Day 4 (Day 4, Learn) and Day 6 (Day 6, Learn) as well as for the Reversal Phase target platform on Day 6 (Day 6, Rev) are shown. * $p < 0.05$.

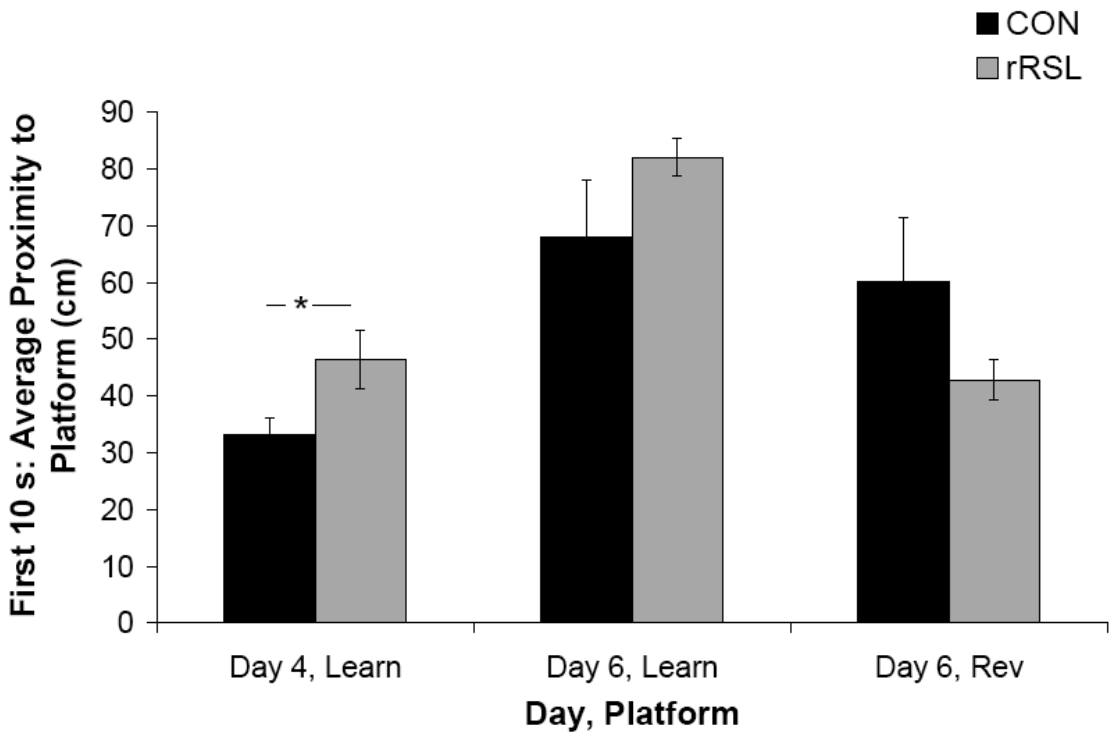


Figure 4.3 Average proximity to the target platform during the first 10 s of the Probe trial

For the average proximity to the target platform for the first 10 s of the probe trial, rRSL were significantly worse than CON on Day 4. Data are shown as mean ± SEM for CON (black) and rRSL (grey). Probe trial data for the Learning Phase target platform on Day 4 (Day 4, Learn) and Day 6 (Day 6, Learn) as well as for the Reversal Phase target platform on Day 6 (Day 6, Rev) are shown. * $p < 0.05$.

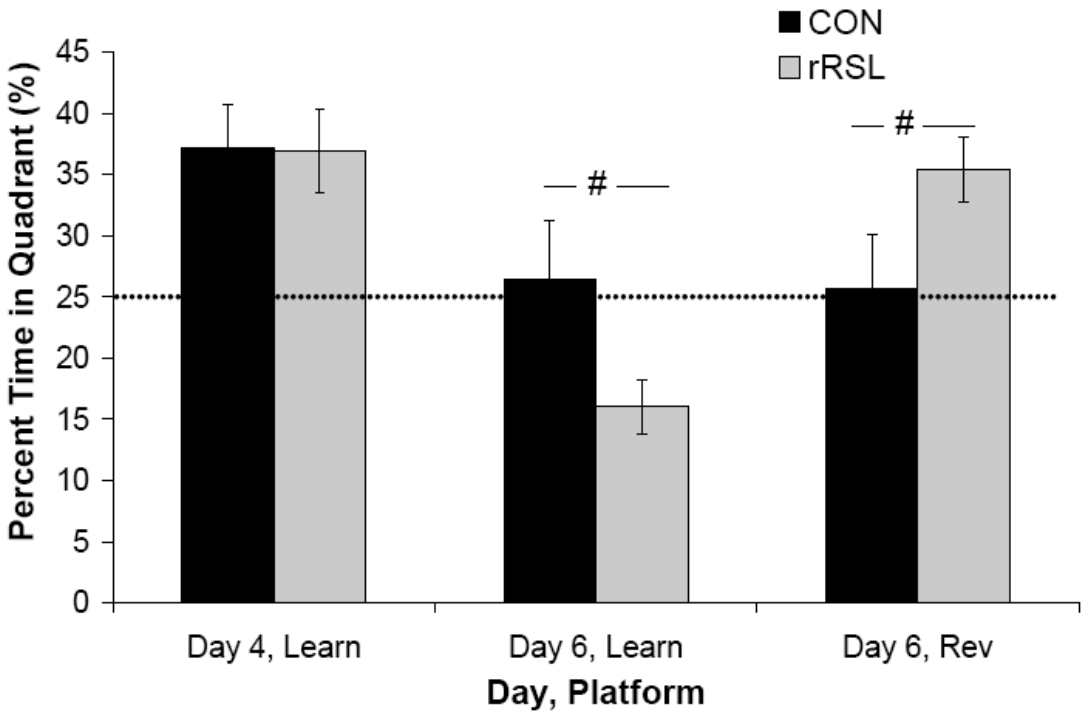


Figure 4.4 Percent time in target quadrant during the 60 s of the Probe trial For the percent time in target quadrant for the entire 60 s probe trial, rRSL were significantly better than CON for the Reversal Phase quadrant on Day 6. Data are shown as mean ± SEM for CON (black) and rRSL (grey). Probe trial data for the Learning Phase target platform on Day 4 (Day 4, Learn) and Day 6 (Day 6, Learn) as well as for the Reversal Phase target platform on Day 6 (Day 6, Rev) are shown. # $p < 0.1$.

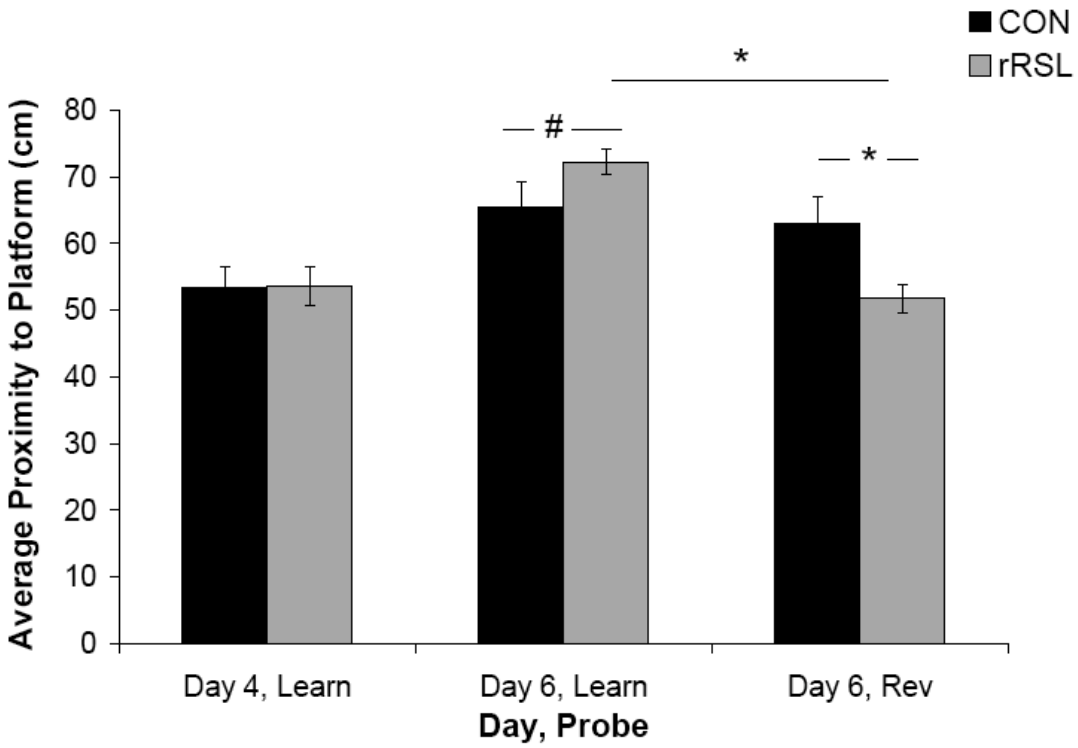


Figure 4.5 Average proximity to the target platform during the 60 s of the Probe trial

For the average proximity to the target platform for the entire 60 s probe trial, RDL were significantly better than CON for the Reversal Phase platform location on Day 6. Data are shown as mean ± SEM for CON (black) and rRSL (grey). Probe trial data for the Learning Phase target platform on Day 4 (Day 4, Learn) and Day 6 (Day 6, Learn) as well as for the Reversal Phase target platform on Day 6 (Day 6, Rev) are shown. On the Day 6 probe trial, controls tended to swim closer to the Learning Phase platform location than previously rRS (rRSL) rats. Previously rRS (rRSL) rats swam significantly closer to the Reversal Phase platform location on the Day 6 probe trial compared to controls. Within group comparisons indicated that rRSL swam significantly closer to the Reversal Phase platform location than the Learning Phase platform location on the Day 6 probe trial. * $p < 0.05$. # $p < 0.1$.

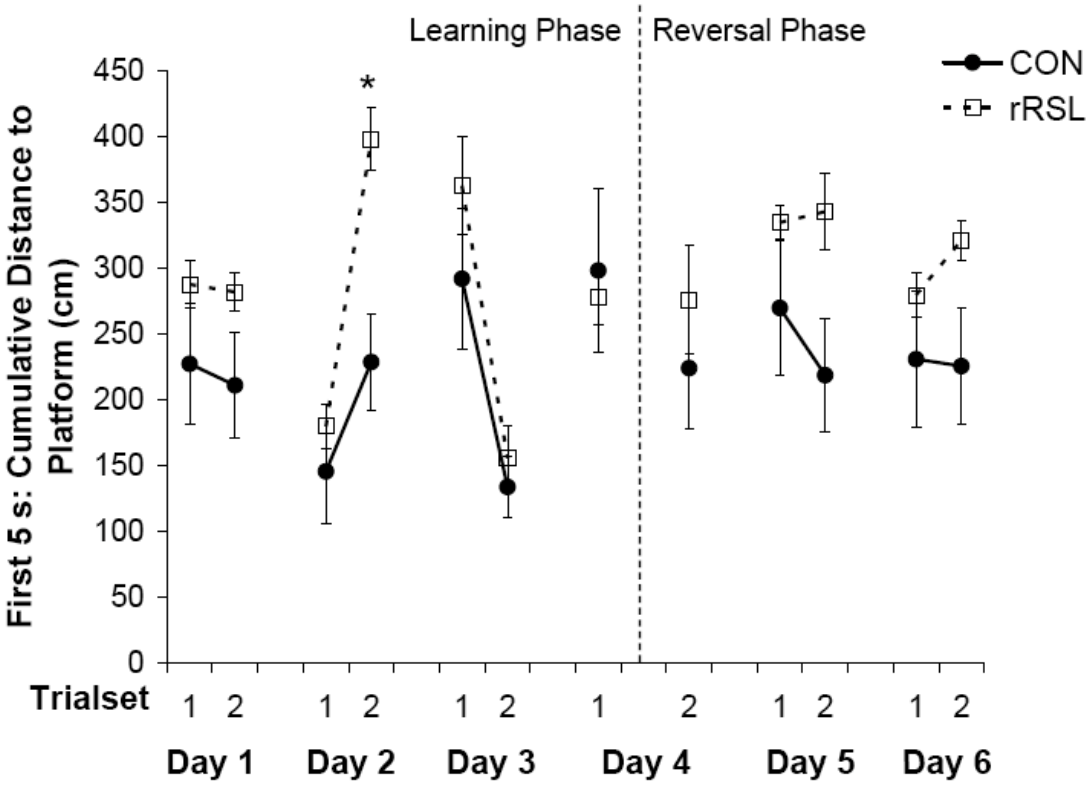


Figure 4.6 The first 5 seconds of cumulative distance from the target platform during the Learning Phase

The first 5 seconds of cumulative distance from the initial spatial learning target platform is shown as mean ± SEM for CON (solid line) and rRSL (dashed line). rRSL were significantly worse than CON on Day 2. * $p < 0.05$.

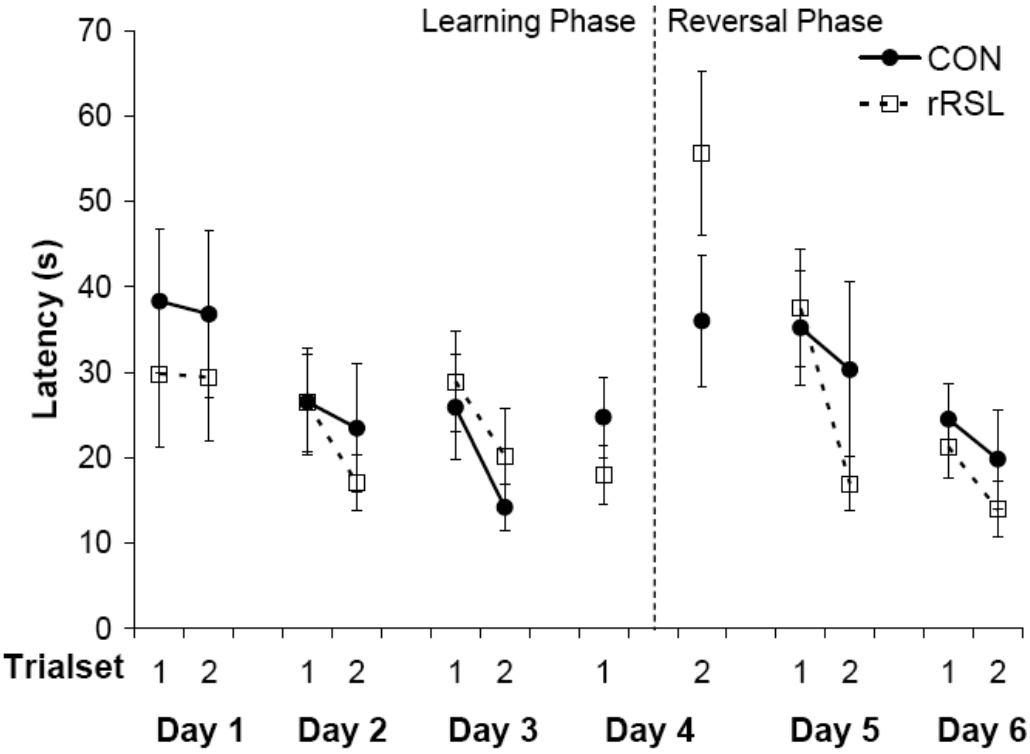


Figure 4.7 Latency to platform
Latency to platform for Day 1 (black striped columns) and Day 2 (grey striped columns) are shown for CON and rRSL. CON significantly improved between Days 1 and 2, while rRSL did not. Data are shown as mean ± SEM.

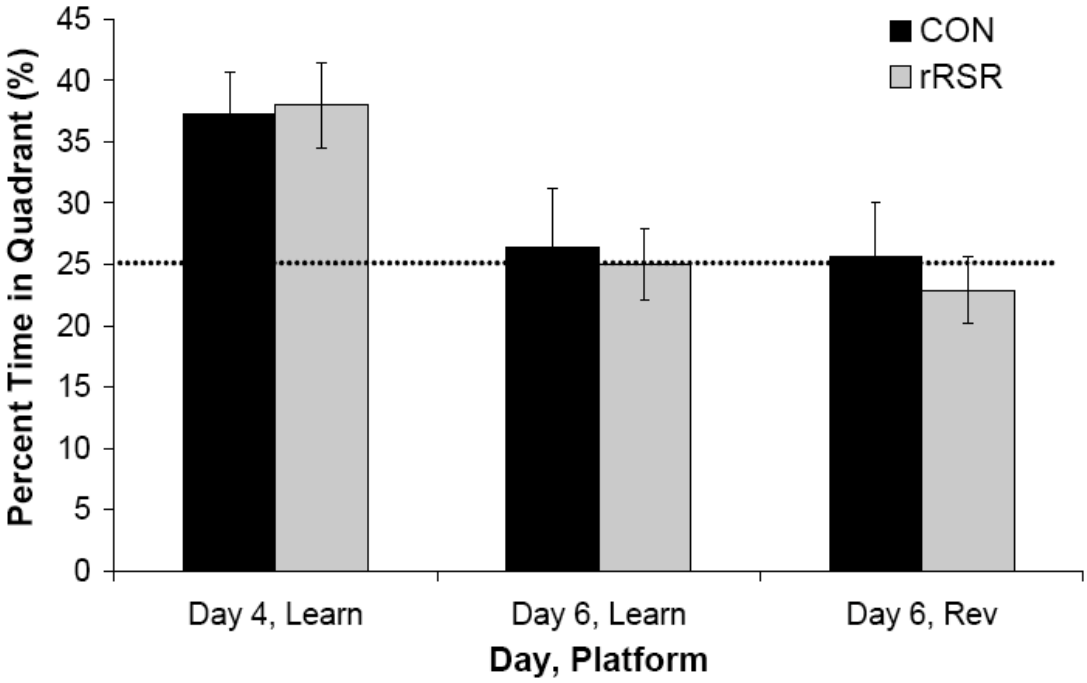


Figure 4.8 Percent time in target quadrant during the 60 s of the Probe trial For the percent time in target quadrant for the entire 60 s probe trial, no group differences were found. Data are shown as mean ± SEM for CON (black) and rRSR (grey). Probe trial data for the Learning Phase target platform on Day 4 (Day 4, Learn) and Day 6 (Day 6, Learn) as well as for the Reversal Phase target platform on Day 6 (Day 6, Rev) are shown.

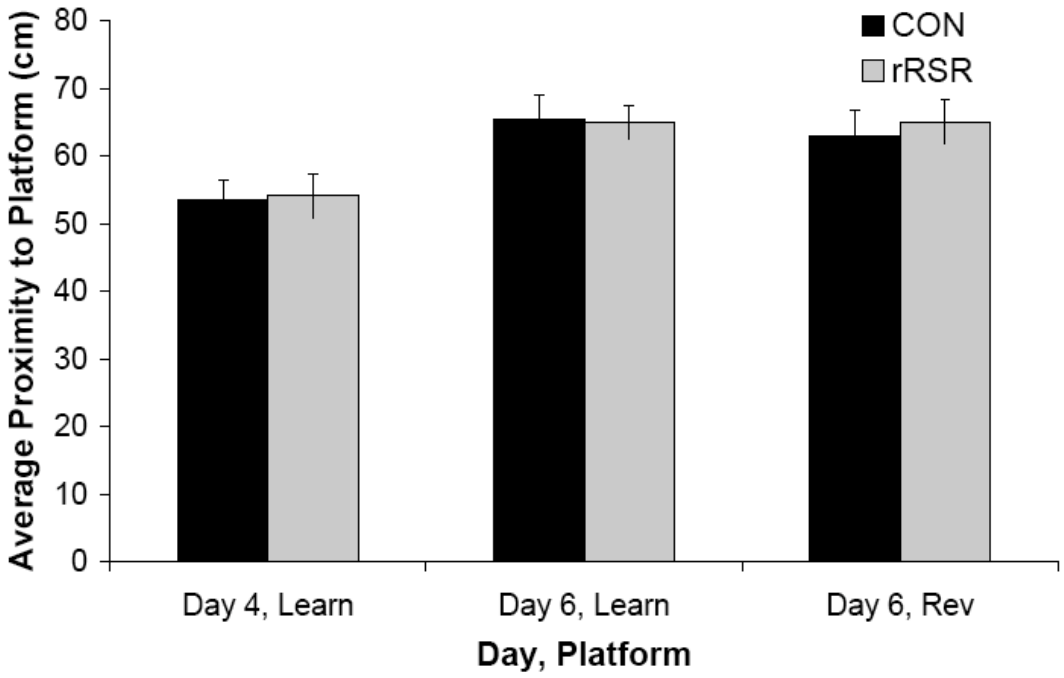


Figure 4.9 Average proximity to the target platform during the 60 s of the Probe trial

For the average proximity to the target platform for the entire 60 s probe trial, no group differences were found. Data are shown as mean ± SEM for CON (black) and rRSR (grey). Probe trial data for the Learning Phase target platform on Day 4 (Day 4, Learn) and Day 6 (Day 6, Learn) as well as for the Reversal Phase target platform on Day 6 (Day 6, Rev) are shown.

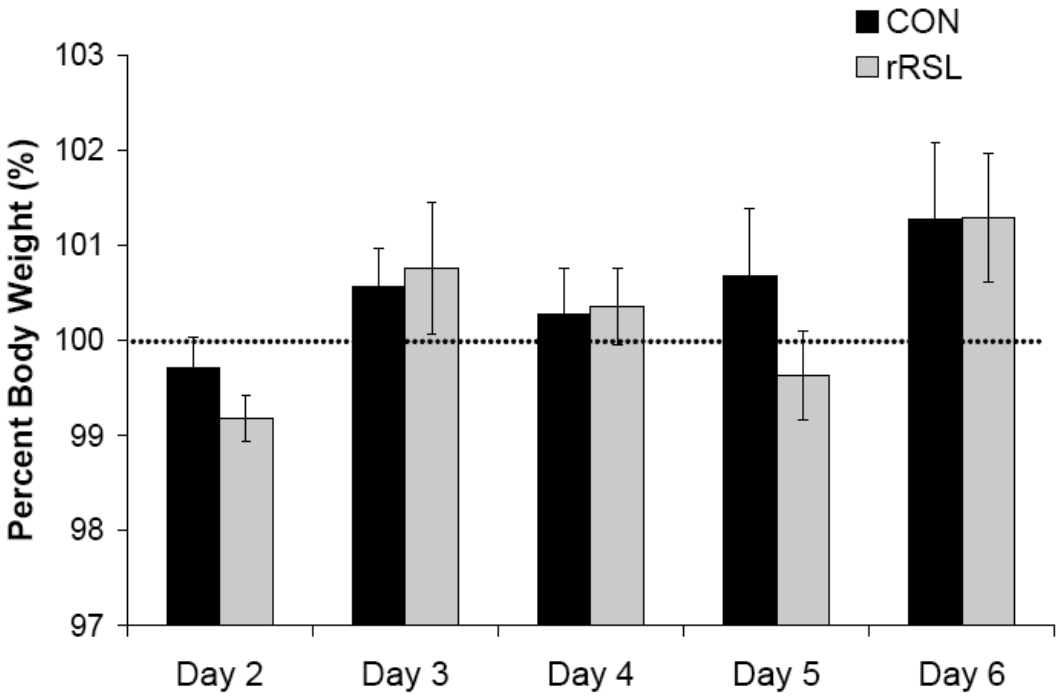


Figure 4.10 Percent body weight based on Day 1 body weights
Body weights are calculated as a percentage of body weight on Day 1. No group differences were found. Data are shown as mean ± SEM for CON (black) and rRSR (grey).

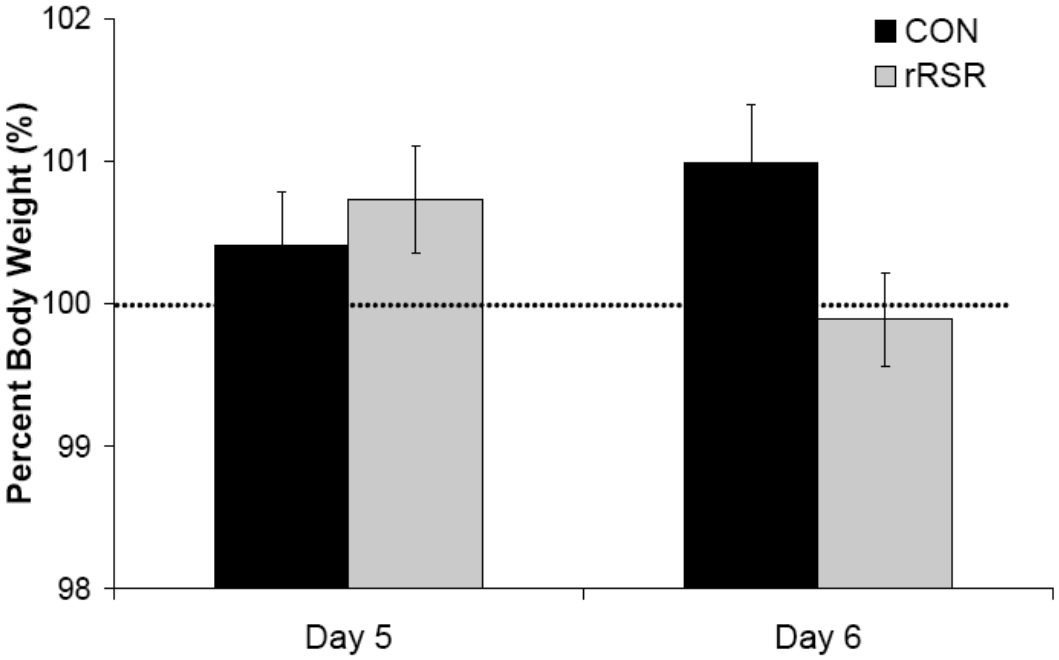


Figure 4.11 Percent body weight based on Day 4 body weights
Body weights are calculated as a percentage of body weight on Day 4. No group differences were found. Data are shown as mean ± SEM for CON (black) and rRSR (grey).

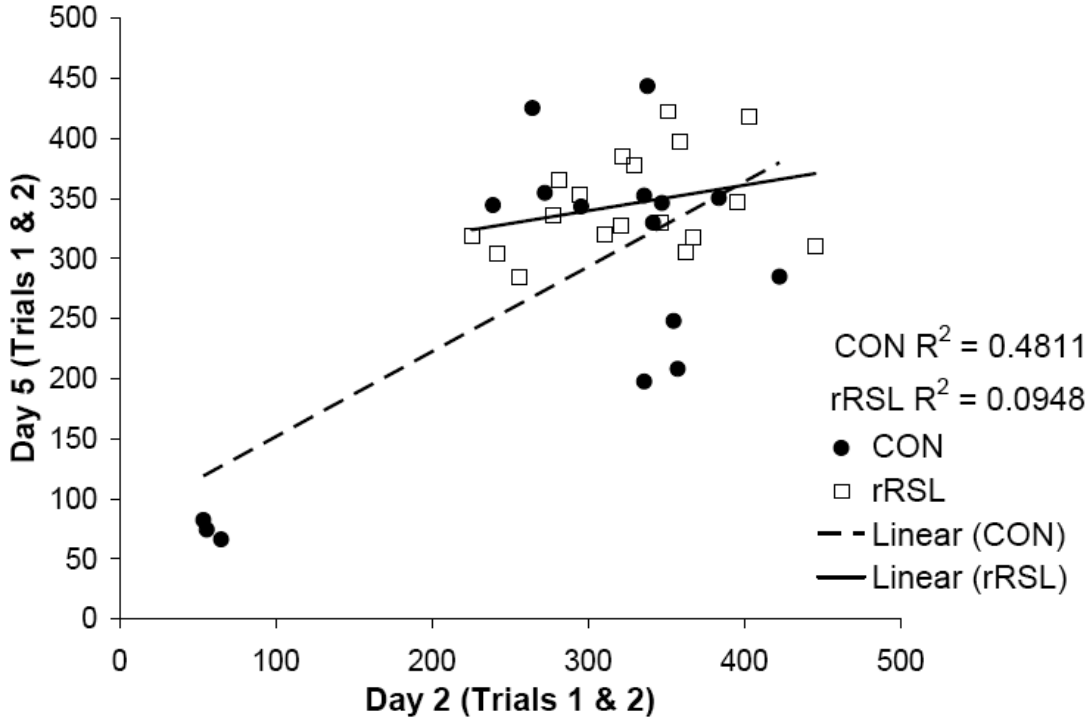


Figure 4.12 Comparison across studies for the first 5 s of cumulative distance

The first 5 s of cumulative distance for the average of trials 1 and 2 on Day 2 are plotted against the average of trials 1 and 2 on Day 5. Data are shown for controls (CON, collapsed across both the 12 TpD and 4 TpD studies, closed circles), and for animals RS restricted during the Learning Phase (rRSL, collapsed across both the 12 TpD and 4 TpD studies, open squares). R^2 values are shown for both controls and rRSL.

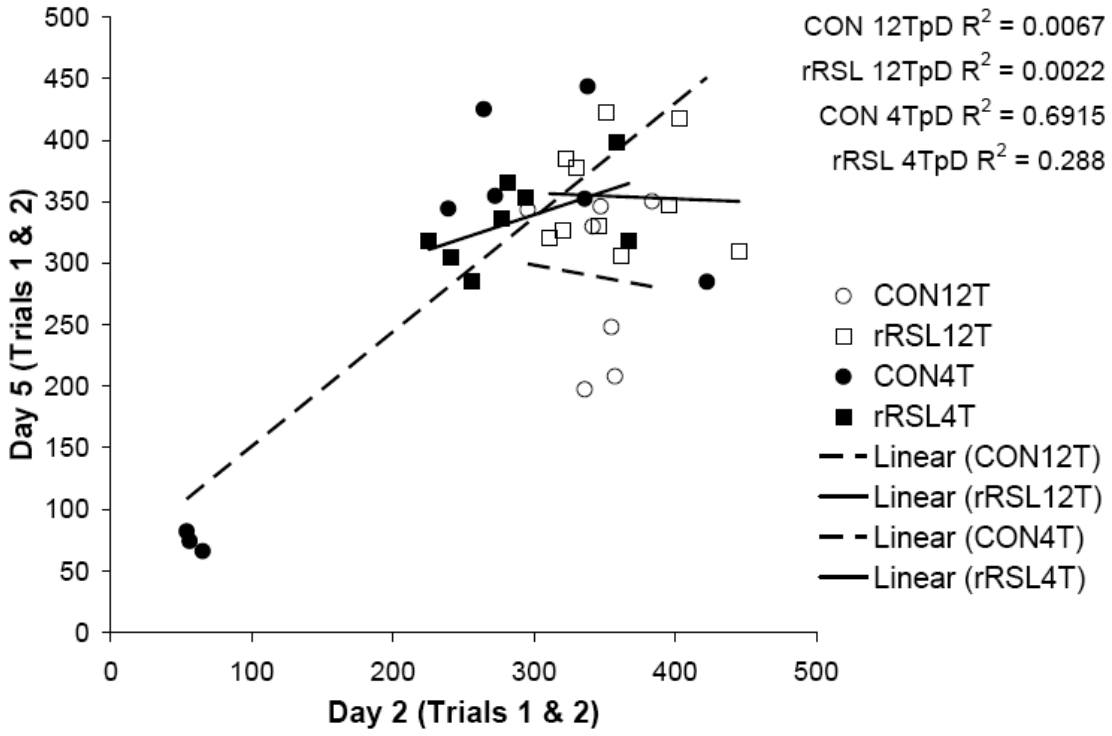


Figure 4.13 Comparison across studies for the first 5 s of cumulative distance

The first 5 s of cumulative distance for the average of trials 1 and 2 on Day 2 are plotted against the average of trials 1 and 2 on Day 5. Data are shown for 4 TpD controls (CON4T, closed circles), 12 TpD controls (CON12T, open circles), 4 TpD rRSL (rRSL4T, closed squares) and for 12 TpD rRSL (rRSL12T, open squares). R^2 values for all groups.

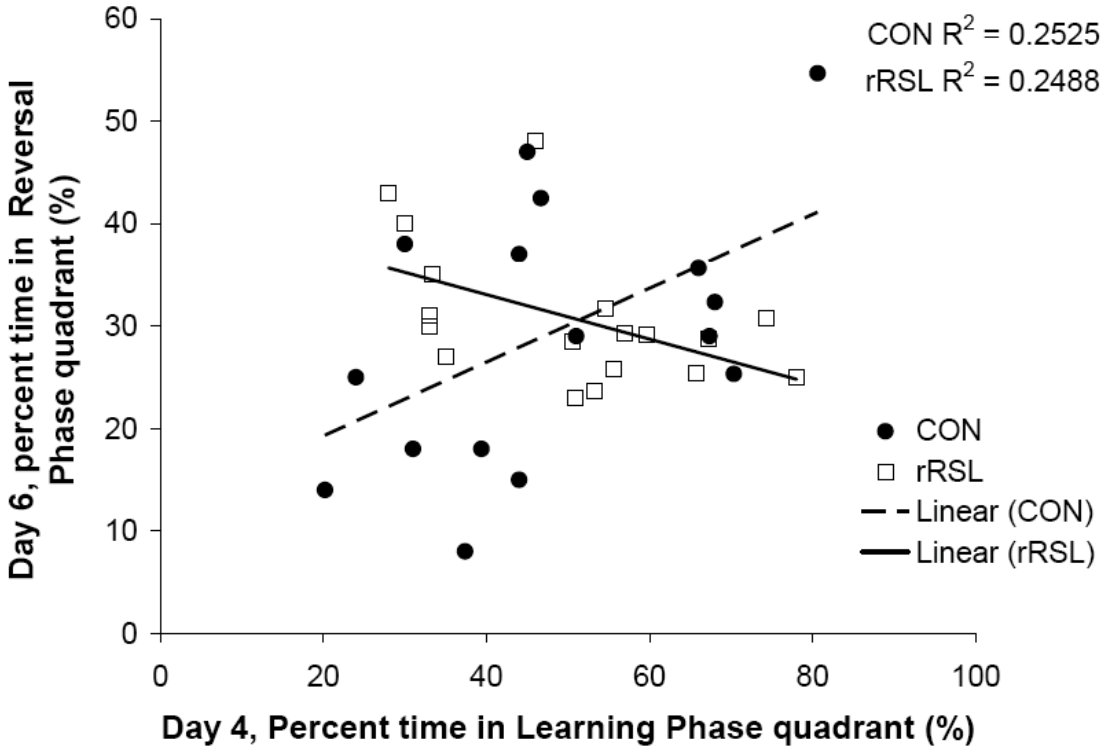


Figure 4.14 Comparison across studies for time spent in target quadrant during the probe trials

Time spent in target quadrant for the Learning Phase quadrant on the Day 4 probe trial and the Reversal Phase quadrant on the Day 6 probe trial are compared to each other. Data are shown for controls (CON, collapsed across both the 12 TpD and 4 TpD studies, closed circles), and for animals RS restricted during the Learning Phase (rRSL, collapsed across both the 12 TpD and 4 TpD studies, open squares). R^2 values are shown for both controls and rRSL. The correlation was significant for both controls ($p < 0.05$) and rRSL ($p < 0.01$). These data indicate that performance on the Learning Phase is associated with performance on the subsequent Reversal Phase and that rRS alters this relationship.

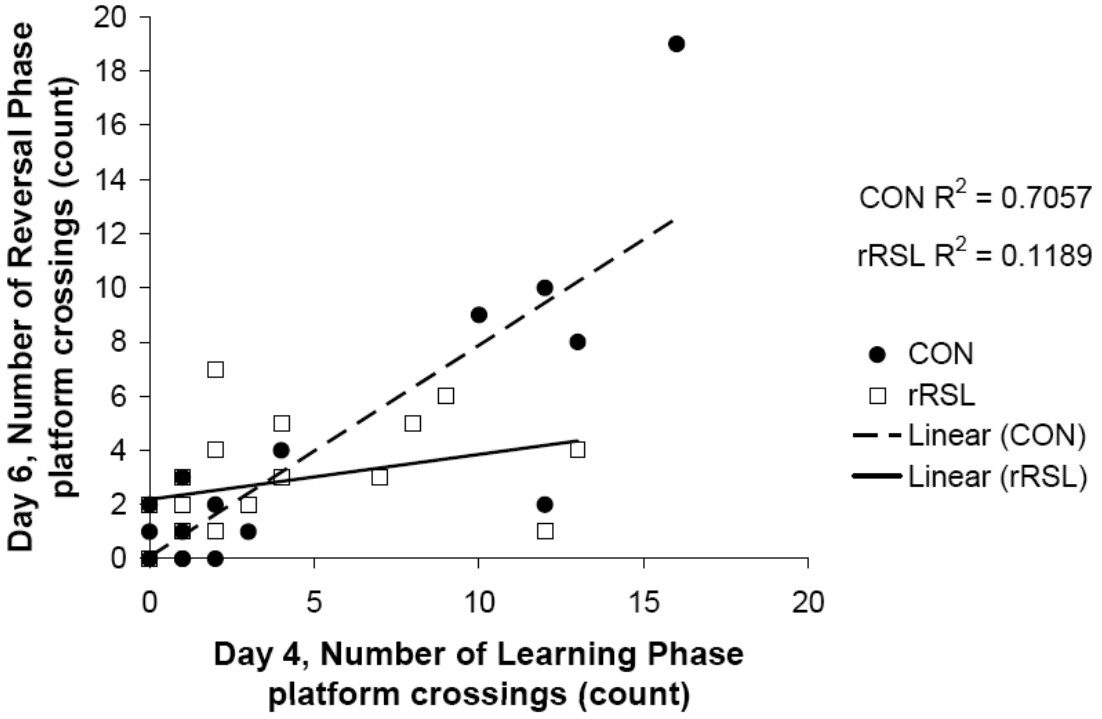


Figure 4.15 Comparison across studies for the number of platform crossings during the probe trials

The number of Learning Phase platform crossings on the Day 4 probe trial and the number of Reversal Phase platform crossings on the Day 6 probe trial are compared to each other. Data are shown for controls (CON, collapsed across both the 12 TpD and 4 TpD studies, closed circles), and for animals RS restricted during the Learning Phase (rRSL, collapsed across both the 12 TpD and 4 TpD studies, open squares). R^2 values are shown for both controls and rRSL.

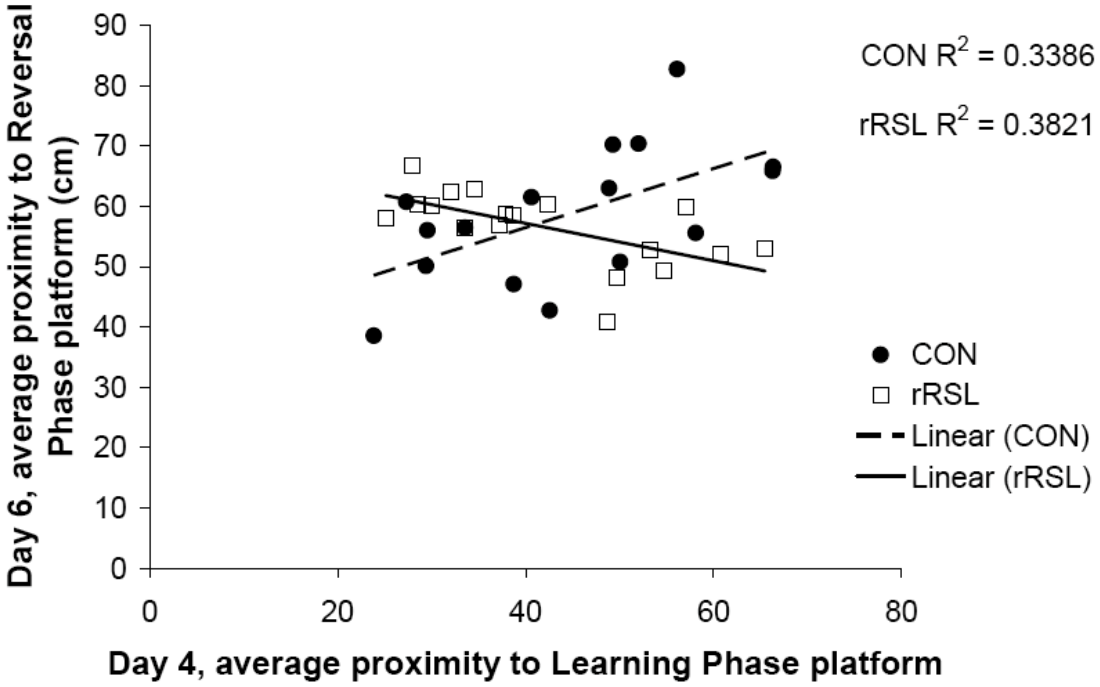


Figure 4.16 Comparison across studies for average proximity to platform during the probe trials

Average proximity to the Learning Phase platform location on the Day 4 probe trial and the Reversal Phase platform location on the Day 6 probe trial are compared to each other. Data are shown for controls (CON, collapsed across both the 12 TpD and 4 TpD studies, closed circles), and for animals RS restricted during the Learning Phase (rRSL, collapsed across both the 12 TpD and 4 TpD studies, open squares). R^2 values are shown for both controls and rRSL. The correlation was significant for both controls ($p < 0.05$) and rRSL ($p < 0.01$). These data indicate that performance on the Learning Phase is associated with performance on the subsequent Reversal Phase and that rRS alters this relationship.

Table 4.1 A Summary of the behavioral results for rRSL compared to controls for the Learning Phase

	Learning Phase
Training	
Latency	* Smith analysis: CON improved, rRSL no improvement
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	* Day 2 CON better than rRSL
Swim Speed	-
Probe Trial	
60 s Percent time in Learning Phase Quadrant	-
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	-
60 s Avg. Proximity to Reversal Phase Platform	-
10 s Probe Percent time in target quadrant	* L.P. CON better than rRSL
10 s Number of platform crossings	-
10 s Probe Avg Proximity to target platform	* L.P. CON better than rRSL
Swim Speed	-

Table 4.1 B Summary of the behavioral results for rRSL compared to controls for the subsequent Reversal Phase

	Reversal Phase
Training	
Latency	* Smith: rRSL improved, CON no improvement
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	-
Swim Speed	-
Probe Trial	
60 s Percent time in Learning Phase Quadrant	# L.P. CON more percent time than rRSL
60 s Percent time in Reversal Phase Quadrant	# R.P. rRSL more percent time than CON
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	# L.P. CON swam closer than rRSL
60 s Avg. Proximity to Reversal Phase Platform	* R.P. rRSL swam closer than CON
10 s Probe Percent time in target quadrant	-
10 s Number of platform crossings	-
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

L.P. Learning Phase, R.P. Reversal Phase. * $p < 0.05$. # $p < 0.1$

Table 4.2 Summary of the behavioral results for rRSR compared to controls for the Reversal Phase

	Reversal Phase
Training	
Latency	-
Pathlength	-
Cumulative Distance	-
5s Cumulative Distance	-
Swim Speed	-
Probe Trial	
60 s Percent time in Learning Phase Quadrant	-
60 s Percent time in Reversal Phase Quadrant	-
60 s Number of Learning Phase Platform Crossings	-
60 s Number of Reversal Phase Platform Crossings	-
60 s Avg. Proximity to Learning Phase Platform	-
60 s Avg. Proximity to Reversal Phase Platform	-
10 s Probe Percent time in target quadrant	-
10 s Number of platform crossings	-
10 s Probe Avg Proximity to target platform	-
Swim Speed	-

L.P. Learning Phase, R.P. Reversal Phase. * $p < 0.05$. # $p < 0.1$

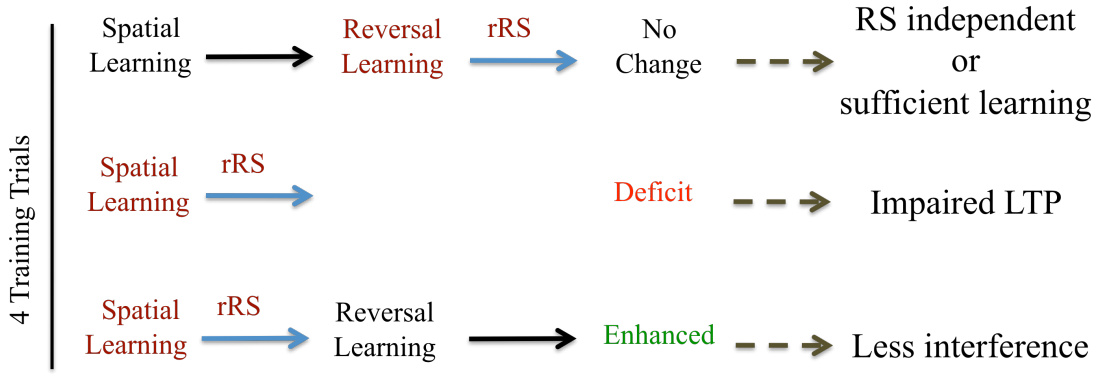


Figure 4.17 Summary of results of 4 training trials per day study. Results are shown for my 4 training trials per day study when rRS was administered during reversal learning (burgundy) and during the initial spatial learning (burgundy). Subsequent reversal learning (black) is also shown. rRS concurrent with the Reversal Phase did not result in performance differences, possibly as a result of sufficient learning prior to the RS manipulation or due to concurrent reversal learning being RS independent. rRS concurrent with the Learning Phase resulted in performance deficits (red) potentially due to an impairment of LTP not forming a stable map of the platform location during the rRS periods. Performance enhancements (green) were observed in previously rRS rats during subsequent reversal learning in comparison to controls. This may be the result of decreased interference, due to prior LTP impairment not forming a stable map, therefore making it easier to reverse to a new platform location.

Condition	12 Trials	4 Trials	Driven by
Reversal Learning	No Change	No Change	RS independent or sufficient learning
Spatial Learning	No Change	Deficit	LTP / depotentiation balance and sufficient learning
Subsequent Reversal Learning	Deficit	Enhanced	Interference

Figure 4.18 Summary of Discussion
Results and theories are presented for both my current study on 4 trials (3rd column) and my previous work using 12 trials of training per day (2nd column). Deficits (red), enhancements (green) and no effects (black) are indicated for concurrent rRS (burgundy) and when in undisturbed sleep (black). Subsequent reversal learning lists the results for rats previously rRS during initial spatial learning in comparison to controls. Theoretical interpretations of the data are shown in the 4th column (Driven by).

References

Beaulieu, I. and R. Godbout (2000). "Spatial learning on the Morris Water Maze Test after a short-term paradoxical sleep deprivation in the rat." Brain Cogn 43(1-3): 27-31.

Bjorness, T. E., B. T. Riley, et al. (2005). "REM restriction persistently alters strategy used to solve a spatial task." Learn Mem 12(3): 352-359.

Blokland, A., J. de Vente, et al. (1999). "Local inhibition of hippocampal nitric oxide synthase does not impair place learning in the Morris water escape task in rats." Eur J Neurosci 11(1): 223-232.

Bodnoff, S. R., A. G. Humphreys, et al. (1995). "Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats." J Neurosci 15(1 Pt 1): 61-69.

Bramham, C. R., C. Maho, et al. (1994). "Suppression of long-term potentiation induction during alert wakefulness but not during 'enhanced' REM sleep after avoidance learning." Neuroscience 59(3): 501-509.

Cimadevilla, J. M. and J. L. Arias (2008). "Different vulnerability in female's spatial behaviour after unilateral hippocampal inactivation." Neurosci Lett 439(1): 89-93.

Cirulli, F., A. Berry, et al. (2000). "Intracerebroventricular administration of brain-derived neurotrophic factor in adult rats affects analgesia and spontaneous behaviour but not memory recall in a Morris Water Maze task." Neurosci Lett 287(3): 207-210.

Cirulli, F., A. Berry, et al. (2004). "Intrahippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze." Hippocampus 14(7): 802-807.

Conrad, C. D., L. A. Galea, et al. (1996). "Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment." Behav Neurosci 110(6): 1321-1334.

Conrad, C. D. and E. J. Roy (1993). "Selective loss of hippocampal granule cells following adrenalectomy: implications for spatial memory." J Neurosci 13(6): 2582-2590.

Cui, R., B. Li, et al. (2007). "Differential effects of psychological and physical stress on the sleep pattern in rats." Acta Med Okayama 61(6): 319-327.

Davis, C. J., J. W. Harding, et al. (2003). "REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus." Brain Res 973(2): 293-297.

Fishbein, W., C. Kastaniotis, et al. (1974). "Paradoxical sleep: prolonged augmentation following learning." Brain Res 79(1): 61-75.

Foy, M. R., M. E. Stanton, et al. (1987). "Behavioral stress impairs long-term potentiation in rodent hippocampus." Behav Neural Biol 48(1): 138-149.

Fu, J., P. Li, et al. (2007). "Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats." Neuroscience 144(4): 1186-1192.
Fujihara, H., R. Serino, et al. (2003). "Six-hour selective REM sleep deprivation increases the expression of the galanin gene in the hypothalamus of rats." Brain Res Mol Brain Res 119(2): 152-159.
Gallagher, M., R. Burwell, et al. (1993). "Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze." Behav Neurosci 107(4): 618-626.
Guzowski, J. F., B. Setlow, et al. (2001). "Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268." J Neurosci 21(14): 5089-5098.
Harrell, L. E., T. S. Barlow, et al. (1984). "Facilitated reversal learning of a spatial-memory task by medial septal injections of 6-hydroxydopamine." Exp Neurol 85(1): 69-77.
Hasselmo, M. E., C. Bodelon, et al. (2002). "A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning." Neural Comput 14(4): 793-817.
Hicks, R. A., A. Okuda, et al. (1977). "Depriving rats of REM sleep: the identification of a methodological problem." Am J Psychol 90(1): 95-102.
Hobson, J. A. and E. F. Pace-Schott (2002). "The cognitive neuroscience of sleep: neuronal systems, consciousness and learning." Nat Rev Neurosci 3(9): 679-693.
Hodges, H. (1996). "Maze procedures: the radial-arm and water maze compared." Brain Res Cogn Brain Res 3(3-4): 167-181.
Hoh, T., J. Beiko, et al. (1999). "Complex behavioral strategy and reversal learning in the water maze without NMDA receptor-dependent long-term potentiation." J Neurosci 19(10): RC2.
Holscher, C., R. Anwyl, et al. (1997). "Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can Be depotentiated by stimulation on the negative phase in area CA1 in vivo." J Neurosci 17(16): 6470-6477.
Ishikawa, A., Y. Kanayama, et al. (2006). "Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus." Eur J Neurosci 24(1): 243-248.
Iwakiri, H., K. Matsuyama, et al. (1993). "Extracellular levels of serotonin in the medial pontine reticular formation in relation to sleep-wake cycle in cats: a microdialysis study." Neurosci Res 18(2): 157-170.
Jouvet, D., P. Vimont, et al. (1964). "[Study of Selective Deprivation of the Paradoxal Phase of Sleep in the Cat.]." J Physiol (Paris) 56: 381.
Joyal, C. C., C. Strazielle, et al. (2001). "Effects of dentate nucleus lesions on spatial and postural sensorimotor learning in rats." Behav Brain Res 122(2): 131-137.
Katsuki, H., Y. Izumi, et al. (1997). "Noradrenergic regulation of synaptic plasticity in the hippocampal CA1 region." J Neurophysiol 77(6): 3013-3020.

Kemp, A. and D. Manahan-Vaughan (2004). "Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition." Proc Natl Acad Sci U S A 101(21): 8192-8197.
Kemp, A. and D. Manahan-Vaughan (2005). "The 5-hydroxytryptamine4 receptor exhibits frequency-dependent properties in synaptic plasticity and behavioural metaplasticity in the hippocampal CA1 region in vivo." Cereb Cortex 15(7): 1037-1043.
Kim, E. Y., G. S. Mahmoud, et al. (2005). "REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus." Neurosci Lett 388(3): 163-167.
Kim, J. J., J. C. Shih, et al. (1997). "Selective enhancement of emotional, but not motor, learning in monoamine oxidase A-deficient mice." Proc Natl Acad Sci U S A 94(11): 5929-5933.
Krugers, H. J., B. R. Douma, et al. (1997). "Exposure to chronic psychosocial stress and corticosterone in the rat: effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity." Hippocampus 7(4): 427-436.
Lacroix, L., I. White, et al. (2002). "Effect of excitotoxic lesions of rat medial prefrontal cortex on spatial memory." Behav Brain Res 133(1): 69-81.
Leconte, P., E. Hennevin, et al. (1974). "Duration of paradoxical sleep necessary for the acquisition of conditioned avoidance in the rat." Physiol Behav 13(5): 675-681.
Li, S., Y. Tian, et al. (2009). "The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats." Learn Behav 37(3): 246-253.
Louie, K. and M. A. Wilson (2001). "Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep." Neuron 29(1): 145-156.
Machado, R. B., D. C. Hipolide, et al. (2004). "Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery." Brain Res 1004(1-2): 45-51.
Machado, R. B., D. Suchecki, et al. (2006). "Comparison of the sleep pattern throughout a protocol of chronic sleep restriction induced by two methods of paradoxical sleep deprivation." Brain Res Bull 70(3): 213-220.
Maei, H. R., K. Zaslavsky, et al. (2009). "What is the Most Sensitive Measure of Water Maze Probe Test Performance?" Front Integr Neurosci 3: 4.
Mashour, G. A., W. J. Lipinski, et al. (in review). "Isoflurane Anesthesia is not Permissive of Homesotatic Processes related to Rapid Eye Movement Sleep."
Mavanji, V. and S. Datta (2003). "Activation of the phasic pontine-wave generator enhances improvement of learning performance: a mechanism for sleep-dependent plasticity." Eur J Neurosci 17(2): 359-370.
McDermott, C. M., M. N. Hardy, et al. (2006). "Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus." J Physiol 570(Pt 3): 553-565.

McDermott, C. M., G. J. LaHoste, et al. (2003). "Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons." J Neurosci 23(29): 9687-9695.
McDonald, R. J., N. Foong, et al. (2007). "The role of medial prefrontal cortex in context-specific inhibition during reversal learning of a visual discrimination." Exp Brain Res 177(4): 509-519.
McGrath, M. J. and D. B. Cohen (1978). "REM sleep facilitation of adaptive waking behavior: a review of the literature." Psychol Bull 85(1): 24-57.
McLay, R. N., S. M. Freeman, et al. (1998). "Chronic corticosterone impairs memory performance in the Barnes maze." Physiol Behav 63(5): 933-937.
Morris, R. (1984). "Developments of a water-maze procedure for studying spatial learning in the rat." J Neurosci Methods 11(1): 47-60.
Morris, R. G., J. J. Hagan, et al. (1986). "Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function." Q J Exp Psychol B 38(4): 365-395.
Morrison, A. R., L. D. Sanford, et al. (2000). "The amygdala: a critical modulator of sensory influence on sleep." Biol Signals Recept 9(6): 283-296.
Nishida, M. and M. P. Walker (2007). "Daytime naps, motor memory consolidation and regionally specific sleep spindles." PLoS One 2(4): e341.
Park, S. P., F. Lopez-Rodriguez, et al. (1999). "In vivo microdialysis measures of extracellular serotonin in the rat hippocampus during sleep-wakefulness." Brain Res 833(2): 291-296.
Pearlman, C. (1973). "REM sleep deprivation impairs latent extinction in rats." Physiol Behav 11(2): 233-237.
Penalva, R. G., M. Lancel, et al. (2003). "Effect of sleep and sleep deprivation on serotonergic neurotransmission in the hippocampus: a combined in vivo microdialysis/EEG study in rats." Eur J Neurosci 17(9): 1896-1906.
Poe, G. R., D. A. Nitz, et al. (2000). "Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep." Brain Res 855(1): 176-180.
Portas, C. M. and R. W. McCarley (1994). "Behavioral state-related changes of extracellular serotonin concentration in the dorsal raphe nucleus: a microdialysis study in the freely moving cat." Brain Res 648(2): 306-312.
Portell-Cortes, I., M. Marti-Nicolovius, et al. (1989). "Correlations between paradoxical sleep and shuttle-box conditioning in rats." Behav Neurosci 103(5): 984-990.
Pouzet, B., H. Welzl, et al. (1999). "The effects of NMDA-induced retrohippocampal lesions on performance of four spatial memory tasks known to be sensitive to hippocampal damage in the rat." Eur J Neurosci 11(1): 123-140.
Rampin, C., R. Cespuglio, et al. (1991). "Immobilisation stress induces a paradoxical sleep rebound in rat." Neurosci Lett 126(2): 113-118.
Rauchs, G., B. Desgranges, et al. (2005). "The relationships between memory systems and sleep stages." J Sleep Res 14(2): 123-140.

Ravassard, P., B. Pachoud, et al. (2009). "Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus." Sleep 32(2): 227-240.
Rechtschaffen, A., B. M. Bergmann, et al. (1999). "Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat." Sleep 22(1): 11-31.
Regnard, M. (1881). "Sleep and Somnambulisnm." Science 2(50): 258-262.
Ribeiro, S., V. Goyal, et al. (1999). "Brain gene expression during REM sleep depends on prior waking experience." Learn Mem 6(5): 500-508.
Ribeiro, S., C. V. Mello, et al. (2002). "Induction of hippocampal long-term potentiation during waking leads to increased extrahippocampal zif-268 expression during ensuing rapid-eye-movement sleep." J Neurosci 22(24): 10914-10923.
Romcy-Pereira, R. and C. Pavlides (2004). "Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP." Eur J Neurosci 20(12): 3453-3462.
Ruskin, D. N., K. E. Dunn, et al. (2006). "Eliminating the adrenal stress response does not affect sleep deprivation-induced acquisition deficits in the water maze." Life Sci 78(24): 2833-2838.
Shouse, M. N., R. J. Staba, et al. (2000). "Monoamines and sleep: microdialysis findings in pons and amygdala." Brain Res 860(1-2): 181-189.
Silvestri, A. J. (2005). "REM sleep deprivation affects extinction of cued but not contextual fear conditioning." Physiol Behav 84(3): 343-349.
Smith, C. (1985). "Sleep states and learning: a review of the animal literature." Neurosci Biobehav Rev 9(2): 157-168.
Smith, C. (1995). "Sleep states and memory processes." Behav Brain Res 69(1-2): 137-145.
Smith, C. and S. Butler (1982). "Paradoxical sleep at selective times following training is necessary for learning." Physiol Behav 29(3): 469-473.
Smith, C. and L. Lapp (1986). "Prolonged increases in both PS and number of REMS following a shuttle avoidance task." Physiol Behav 36(6): 1053-1057.
Smith, C. and G. M. Rose (1996). "Evidence for a paradoxical sleep window for place learning in the Morris water maze." Physiol Behav 59(1): 93-97.
Smith, C. and G. M. Rose (1997). "Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze." Behav Neurosci 111(6): 1197-1204.
Smith, C. and P. T. Wong (1991). "Paradoxical sleep increases predict successful learning in a complex operant task." Behav Neurosci 105(2): 282-288.
Smith, C., J. Young, et al. (1980). "Prolonged increases in paradoxical sleep during and after avoidance-task acquisition." Sleep 3(1): 67-81.
Smith, C. T., J. M. Conway, et al. (1998). "Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task." Neurobiol Learn Mem 69(2): 211-217.

Stickgold, R. and M. P. Walker (2005). "Sleep and memory: the ongoing debate." Sleep 28(10): 1225-1227.
Suchecki, D., B. Duarte Palma, et al. (2000). "Sleep rebound in animals deprived of paradoxical sleep by the modified multiple platform method." Brain Res 875(1-2): 14-22.
Suchecki, D., L. L. Lobo, et al. (1998). "Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation." J Sleep Res 7(4): 276-281.
Suchecki, D., P. A. Tiba, et al. (2002). "Hormonal and behavioural responses of paradoxical sleep-deprived rats to the elevated plus maze." J Neuroendocrinol 14(7): 549-554.
Suchecki, D. and S. Tufik (2000). "Social stability attenuates the stress in the modified multiple platform method for paradoxical sleep deprivation in the rat." Physiol Behav 68(3): 309-316.
Sullivan, R. M. and A. Gratton (2002). "Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent." Brain Res 927(1): 69-79.
Tse, D., R. F. Langston, et al. (2007). "Schemas and memory consolidation." Science 316(5821): 76-82.
Underwood, B. J. (1957). "Interference and forgetting." Psychol Rev 64(1): 49-60.
van Hulzen, Z. J. and A. M. Coenen (1981). "Paradoxical sleep deprivation and locomotor activity in rats." Physiol Behav 27(4): 741-744.
Venkatakrishna-Bhatt, H., J. Bures, et al. (1979). "Paradoxical sleep deprivation retards extinction of conditioned taste aversion." Behav Neural Biol 25(1): 133-137.
Vertes, R. P. (2004). "Memory consolidation in sleep; dream or reality." Neuron 44(1): 135-148.
Vertes, R. P., & Eastman, K. E. (2000). The case against memory consolidation in REM sleep. *Behav Brain Sci, 23*(6), 867-876; discussion 904-1121.
Vertes, R. P. and J. M. Siegel (2005). "Time for the sleep community to take a critical look at the purported role of sleep in memory processing." Sleep 28(10): 1228-1229; discussion 1230-1223.
Wagner, U., S. Gais, et al. (2004). "Sleep inspires insight." Nature 427(6972): 352-355.
Wang, G. P., L. Q. Huang, et al. (2009). "Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation." Neuroreport 20(13): 1172-1176.
Whishaw, I. Q. and J. Tomie (1997). "Perseveration on place reversals in spatial swimming pool tasks: further evidence for place learning in hippocampal rats." Hippocampus 7(4): 361-370.
Yang, H. W., Y. W. Lin, et al. (2002). "Change in bi-directional plasticity at CA1 synapses in hippocampal slices taken from 6-hydroxydopamine-treated rats: the role of endogenous norepinephrine." Eur J Neurosci 16(6): 1117-1128.
Yoo, S. S., P. T. Hu, et al. (2007). "A deficit in the ability to form new human memories without sleep." Nat Neurosci 10(3): 385-392.

Youngblood, B. D., J. Zhou, et al. (1997). "Sleep deprivation by the "flower pot" technique and spatial reference memory." Physiol Behav 61(2): 249-25

Chapter 5

General Discussion

Summary

Overall, my results suggest that memory consolidation of initial spatial learning and subsequent learning are affected by an interaction between the amount and timing of RS, the method of RD and the learning load. My results also suggest that reversal learning, although a spatial learning task, is not affected by concurrent rRS although it is affected by prior rRS and learning experience.

In my studies I found that with 12 trials of training per day in the Morris water maze, concurrent rRS did not affect performance during initial spatial learning, nor did it affect concurrent reversal learning (Chapter 2). However, rRS during initial learning did result in performance deficits during subsequent reversal training (Chapter 2). Animals RS deprived during initial spatial training later appeared less flexible toward learning the new platform, or more strongly recalled the original platform, continuing to prefer the initial spatial learning

platform location even by the Day 6 probe trial following a total of 18 trials of reversal learning. This result indicates that under intensive training conditions RS in the two time windows tested during the Reversal Phase did not affect consolidation, but rRS in the 1st 6 hr window did impair subsequent learning abilities when administered during the Learning Phase.

I also found that rRS with a high water level within the deprivation chamber did not result in performance deficits during either concurrent initial spatial learning or later reversal learning as compared to controls (Chapter 3). In the comparison between rats rRS with a high versus a low level of water in the deprivation chamber, no performance differences were identified during initial spatial learning, though on the Day 6 probe trial, animals previously RS deprived with high water in the deprivation chamber preferred the reversal learning platform location more than rats RS deprived with low water (Chapter 3). This result agrees with the conclusions drawn from Chapter 2 and shows that the RD method that produced more RS rebound (also used in Chapter 2) was associated with the impairments on later reversal learning.

Lastly, I found that with a lighter training load, only 4 training trials per day, concurrent with rRS, initial spatial learning performance was impaired whereas the more intensive training sessions of Chapters 2 and 3 had not revealed this RD-associated initial spatial learning deficit. Further, while rRS applied concurrent with reversal learning had no effect, previous rRS resulted in

performance enhancements during subsequent reversal learning. The results from this chapter indicate that the same training and RD procedures that impaired initial spatial learning also assist later consolidation of new information introduced after the rRS period is over.

In Chapter 4, I proposed a theoretical model (Figure 4.17) to explain the relationship between learning load and rRS based on the results from my studies and findings in the literature. It appears from my studies that if sufficient learning occurs prior to rRS, learning consolidation occurs independent of RS (rRS concurrent with: 12 trial Learning Phase and both 12 and 4 trial Reversal Phase resulted in no performance deficits). However, subsequent reversal learning can be affected by prior exposure to rRS. Based on previously published findings (e.g. Kim, et al., 1997; Poe, Nitz, McNaughton, & Barnes, 2000), my results could represent a delay in the removal of the representation of the prior learning from the hippocampus or an offset in the LTP / depotentiation balance. It is possible that the performance deficits measured during subsequent reversal learning (12 training trial per day study, Chapter 2) result from the Learning Phase platform location remaining novel in its representation within the hippocampus. Therefore, when the new platform location was to be learned, there may have been increased proactive inhibition through competition of the two platform locations. Further the effects of prior rRS on subsequent reversal learning when the rats' sleep is undisturbed may be due to decreased flexibility in learning resulting from the prior rRS.

On the other hand, if insufficient learning occurs prior to the RS manipulation (rRS concurrent with 4 trial Learning Phase), then its consolidation seems to remain RS dependent. When rRS is concurrent with fewer training trials, LTP may be impaired, as seen with previous LTP and rRS experiments (Romcy-Pereira & Pavlides, 2004). As hippocampal-dependent consolidation may not have occurred either on-line or during the subsequent rRS period, rats may rely on hippocampal independent strategies. A change in strategy from hippocampal dependent to independent has been previously reported following rRS (Bjorness, Riley, Tysor, & Poe, 2005). Although non-hippocampal and hippocampal dependent strategies typically interfere with each other, it is possible that reversal of spatial learning is not a pure hippocampus dependent task. Therefore, perfected use of the non-hippocampal dependent strategies across the 4 TpD Learning Phase concurrent with rRS could facilitate the subsequent reversal learning. Alternatively if a rat had a less stable map of the initial learned platform location there may be less interference possibly leading to a reduced level of proactive interference (due to inadequate hippocampal based learning of the Learning Phase platform location) which could also result in performance enhancements during subsequent reversal learning as observed with 4 training trials per day

RS effects on initial Spatial Learning

To date the Smith and Rose (C. Smith & Rose, 1996, 1997) studies have been considered among the key papers indicating the relevance of REM sleep for learning in rodents. Indeed, reviews and debates on this topic heavily cite these references as the representative animal literature showing evidence for the dependence of learning on REM sleep (e.g.Peigneux, et al., 2003; Stickgold & Walker, 2005). Smith and Rose's work (C. Smith & Rose, 1996, 1997) were the only studies focused on short bouts of REM sleep deprivation or RS restriction concurrent with training in the Morris water maze. Until my current work, more than 10 years after the work of Smith and Rose (Beaulieu & Godbout, 2000; Bjorness, et al., 2005; Le Marec, Beaulieu, & Godbout, 2001; C. Smith & Rose, 1996, 1997; C. T. Smith, Conway, & Rose, 1998), few others have reported on the effects of short bouts of REM sleep deprivation with spatial learning.

A serious issue with the established literature focusing on either RD or rRS and learning in the Morris water maze is that the majority of papers have reported latency as their main measure of performance, and therefore learning. However, as has been previously discussed in Chapter 2, latency is a vulnerable measure not truly reporting how well the location of the hidden platform was known (Gallagher, Burwell, & Burchinal, 1993; Hodges, 1996) as it could have been found by chance or by using alternative strategies (e.g. a thigmotaxic search pattern). Further, it has been suggested that movement in rodents is increased following REM sleep deprivation. Increased movement could affect latency to

platform by altering the swim speed of the rodent. Latency provides no indication that the rat has reached the platform due to either knowing its location or finding it per chance with faster swim speeds (and possibly searching more).

While studies using longer bouts of RD (24 – 72 hrs) have included other measures of performance (e.g. Li, et al., 2009; Ruskin, Dunn, Billiot, Bazan, & LaHoste, 2006; Wang, et al., 2009; Youngblood, Zhou, Smagin, Ryan, & Harris, 1997), my studies are the first to report on the effects of RD or rRS in the Morris water maze using the Gallagher measures. Additionally I used a range of other measures for both training (pathlength, latency and swim speed) and probe trials (percent time in target quadrant, number of platform crossings, pathlength and swim speed). Measures other than latency that have been reported in the previous RD or rRS and Morris water maze studies are pathlength (Li, et al., 2009; Wang, et al., 2009), number of target quadrant entries during training (C. Smith & Rose, 1996), area under the curve for both latency and pathlength (Youngblood, et al., 1997) and percent time spent in target quadrant during a probe trial (Wang, et al., 2009). Further, instead of just looking at the entire trial length, I investigated the first 5 s of the training trials to determine if there were differences in the initial direction chosen, an indictor of reference memory. In addition to this I measured the first 10 s alone of the probe trial to again determine if initially there was any effect of rRS on the direction chosen. Thus, the studies presented in this dissertation are more extensive than those

previously done to explore the effects of short periods of RD or rRS on spatial learning in the Morris water maze.

Using rRS via a short bout of RD I found that, similar to Smith and Rose's study (C. Smith & Rose, 1996) with 4 trials per day in the Morris water maze, a short bout of RD resulted in a performance deficit during training on the second day (Chapter 4). However my study indicated a delayed deficit on Day 2 in contrast to Smith and Rose's more immediate deficit shown by a latency to platform (delay) for the first trial on Day 2. Further, though I found no difference in the latency measure, the lack of improvement between Days 1 and 2 for the rRS rats in my study was evident using Gallagher's more sensitive and robust cumulative distance measure. When I studied the effect of a short bout of RD concurrent with 12 trials of training per day in the Morris water maze (Chapter 2), I found no performance deficits. This is in contrast to Smith and Rose's later (C. Smith & Rose, 1997) work. The results from these two chapters (Chapters 2 and 4) on concurrent RD during initial spatial learning, add to the current literature and understanding that was drawn from the Smith and Rose studies (C. Smith & Rose, 1996, 1997), suggesting that the interactive effect of REM sleep and learning may be less robust and clear than generally described by those advocating that RS is important for learning. My data emphasize that a clearer understanding of the role of RS for learning is possible when learning load, type of learning in relation to the rRS period and the RD technique itself are considered.

We first proposed that the lack of effect of short bouts of RD on initial spatial learning, seen with 12 training trials per day in Chapter 2, could be a result of the differences in deprivation technique used in our laboratory as compared to other research groups. This was the reason for the study on high versus low water within the deprivation chambers (Chapter 3). We initially thought that the difference in results on the 12 trial studies was because a high level of water within the deprivation chambers (Chapter 3) would result in less specific sleep deprivation and increased levels of stress over the low water level RD technique typically used in our laboratory. However, my sleep and rebound results suggest that this is not the case. Surprisingly, the results from Chapter 3 indicated that RD with low level water led to poorer performance than RD with high level water, which would have predicted that the performance deficits concurrent with short bouts of RD in Chapters 2 and 4 should have been greater than those found in previous studies in the Morris water maze (C. Smith & Rose, 1996, 1997). It is not possible for me to speculate how my results may have differed if longer deprivation bouts had been used, to compare my results to others that have described RD associated deficits in Morris water maze performance (Li, et al., 2009; Ruskin, et al., 2006; Wang, et al., 2009; Youngblood, et al., 1997). However, in general, similar results have been found when comparing short and long bouts of RD in both the spatial learning (Bjorness, et al., 2005; Li, et al., 2009; C. Smith & Rose, 1996, 1997; C. T. Smith, et al., 1998; Wang, et al., 2009) and conditioned bar pressing literature (Pearlman, 1973).

The precise methodologies used with the inverted flowerpot technique when administering RD are variable in the spatial learning and RD literature. Prior to my study in Chapter 3, no comparative studies had been made to determine how the differing level of water used within the deprivation chamber could alter spatial learning performance, nor how the duration spent in various sleep / waking states during the deprivation and post-deprivation (rebound) periods would relate to spatial learning. One limitation of my study was that the animals used to measure the sleep / waking states were not exposed to the learning task prior to the RD session. Thus, I do not know whether training in the Morris water maze combines with the differences in water level within the deprivation chambers to alter sleep. This separation between sleep studied and learning groups limits my ability to directly attribute differences observed in performance in the Morris water maze with changes in the sleep / waking states of the high vs. low water level groups. The differences that were measured in the sleep / waking states associated with the water level (increased RS rebound following RD with low level water 2 – 4 hrs after the deprivation period) do not necessarily account for the observed performance differences in the Morris water maze. The increased RS rebound with low water level could be associated with the observed impaired performance. Whether the performance results from the RS rebound itself or increased pressure for RS as a result of the deprivation period itself.

Further, my study does not concur with theories that RD effects on learning are mediated by stress. While the HW group appear more stressed after the first bout of RD (on Day 2, HW had a significantly higher drop in percent body weight and increased velocity as compared to LW, and both measures can be associated with increased stress levels), performance in the Morris water maze remained unaffected compared with controls, despite the reports that stress disrupts learning (Bodnoff, et al., 1995; Conrad, Galea, Kuroda, & McEwen, 1996; Foy, Stanton, Levine, & Thompson, 1987; Krugers, et al., 1997; McLay, Freeman, & Zadina, 1998). In a study by Ruskin et al. (2006) adrenalectomized rats RD for 72 hrs prior to learning in the Morris water maze, still showed performance deficits in the Morris water maze, suggesting that RD itself is responsible for the performance deficits rather than stress associated with the deprivation technique. Thus both my study and Ruskin's deemphasize the role of stress in learning concurrent with RD.

Another potential reason for the differences between the Smith and Rose studies (C. Smith & Rose, 1996, 1997) and my own may be the result of using only one inverted flowerpot in their studies (C. Smith & Rose, 1996, 1997) and multiple flowerpots in mine (also used in: Bjorness, et al., 2005; Ravassard, et al., 2009). Using only one pot is thought to increase stress as a result of movement restriction (Coenen & van Luijtelaar, 1985), however based on Ruskin's work (Ruskin, et al., 2006) and my own results for HW as compared to LW, such

increased stress would not necessarily result in a performance deficit in the Morris water maze.

Examination of my data from the 12 training trials per day study (Chapter 2) reveals that the performance level at the end of Day 1 reached a near asymptotic level, with little improvement over the remainder of the Learning Phase period. Approaching a ceiling effect in learning prior to the first bout of RD could account for the lack of concurrent RD associated effects (Chapter 2) and the observed effect that when fewer trials were given prior to the first bout of RD during the 4 training trials per day study concurrent rRS associated learning deficits were revealed (Chapter 4). However, Smith and Rose (1997) also used 12 training trials and found a deficit. One difference between our two studies was that the rats in my studies were first tested in the visual form of the Morris water maze (10 trials total, 5 trials over 2 days), while those used in the Smith laboratory were not. Thus, my rats would have been more familiar with the maze, though not the spatial components since they were blocked by a curtain during visual testing, perhaps requiring them to learn less about the maze task (they have previously learned that a platform is always present and some of the procedural strategies to solve the task) and potentially to be less stressed upon their first exposure to the hidden version of the Morris water maze. A further difference between my work and the results from prior studies could also stem from a variable degree of cue-richness in the maze room. With fewer room cues to form a spatial map to find the hidden platform with, the task could be considered more difficult than

navigating a map rich with cues. This cue-deficit associated challenge could in turn affect the number of trials required to learn the location of the hidden platform. The vulnerability of task performance to the effects of RD could depend on the complexity of the task itself to be learned. Such argument would suggest that the spatial room cues used in my studies were more rich than those used in the Smith et al studies, and thus the task more easily mastered within the 12 trials of my protocol.

RS effects on Reversal Learning

To date, no reports have been made on the effects of RD on reversal learning in the Morris water maze. In fact, thus far no reports have been made on RD effects on reversal spatial learning in general. My data using both 4 and 12 daily training trials did not result in any concurrent rRS-associated deficits in reversal learning, even following only 2 reversal training trials on Day 4 (Chapter 4). Although not definitive, my data suggest that the reversal of spatial learning is impervious to the effects of RD. It may be that the reversal learning task is too simple, perhaps again, because of the richness of the room cues, and the room and/or strategies to solve the task is too well known by the time of the reversal trials. In the 12 trial study (Chapter 2), reversal performance on Days 5 and 6 appear equivalent to if not worse than performance on Days 2 and 3, whereas for the 4 trial study (Chapter 4) performance on Days 5 and 6 appear equivalent or better than Days 2 and 3 (latency). As described in Chapter 4, the lack of concurrent rRS initial training deficit during the 12 trial study could be a result of sufficient within-day or

on-line learning on Day 1 prior to the first bout of RD. Similarly, even just 2-6 trials of reversal learning could provide sufficient on-line learning (Day 4 Reversal Phase, Chapter 4) prior to the first bout of RD to protect against the effects of concurrent rRS. My studies indicate that reversal learning is not vulnerable to concurrent rRS after 6 or even 2 trials of training.

As the results for rRS concurrent with reversal learning differ from those with initial spatial learning, these two types of learning may not be comparable. Further research would be required to further address whether the vulnerability differences to rRS in reversal and initial spatial learning are due to over exposure to a similar task or differences in the rRS vulnerability of underlying neural networks. While both initial spatial learning and reversal spatial learning are thought to rely on the dorsal hippocampus, my data indicate that the effects of RD on spatial learning cannot be generalized to reversal spatial learning.

Subsequent Reversal Learning

My dissertation also focuses on the effect of RD on subsequent learning. Again, this is an area lacking in research. There has been some work on the effect of rRS on subsequent extinction of fear conditioning using the rodent model (Silvestri, 2005). Silvestri's work consisted of RS restricting rodents using the inverted flowerpot technique for 6 hrs immediately following fear conditioning (both cued and contextual). Two days later, rats were tested for their response to extinction training. At the start of extinction, all rats responded similarly, however

those that had received RD following cued fear conditioning did not extinguish, while controls did, after repeated exposures to the tone. In contrast, both groups learned to extinguish to the context at a similar rate. This study was the first to indicate that rRS can affect a form of subsequent spatial learning.

In my studies, I found that rats previously deprived of RS during the Learning Phase with 12 trials per day swam closer to the Learning Phase platform location following reversal learning on the Day 6 probe trial. This could indicate either stronger learning during the Learning Phase, which was concurrent with the rRS or that rRS leads to either a future lack of flexibility in learning or to a persistent retention of the originally learned platform location. In contrast, when rats were trained with 4 trials per day, rats previously deprived of RS during the Learning Phase had the complete opposite effect, showing a preference as compared to controls for the Reversal Phase platform location following reversal learning on the Day 6 probe trial. This suggests that prior RD concurrent with fewer trials lead to enhanced subsequent learning. My results thus indicate an interaction between RD and learning load (number of training trials per day) in the Morris water maze. Previous literature has ascribed the potential lack of RD-associated deficit to be the result of too many trials (for review: McGrath & Cohen, 1978), however this is the first study to focus on the effect of the number of trials on rRS-associated learning in the Morris water maze and the effects on subsequent learning. It is possible that the studies indicating that RD has no affect on

learning could have been the result of too much learning prior to RD and that an affect may have been noted if subsequent learning had been investigated.

RS Restriction Windows

Previous work describing an interaction between REM sleep and learning load came from Smith's laboratory (C. Smith & Rose, 1996, 1997) where a 'heavy learning load', 12 training trials in the Morris water maze, was only sensitive to RD immediately following training, while a lighter learning load, 4 training trials in the Morris water maze, was sensitive to a RD period delayed by 4 hrs after training. When I studied the effects of RD on reversal learning, two periods of RD were utilized (Chapter 2), one immediately following training, which was my target group, and a second delayed by 6 hrs, which was my control for the deprivation-associated stressors. As RD appeared to have no effect whether applied early (0 – 6 hrs) or late (6 – 12 hrs) following training, I no longer included the late RD control for my additional studies, using only the immediate RD period when I studied the impact of RD on the initial spatial Learning Phase and subsequent Reversal Phase for both the 12 trial study and the 4 trial study (Chapter 4). Although results from Smith and Rose's work (C. Smith & Rose, 1996) with 4 training trials per day would suggest a RD sensitive period delayed by 4 hrs, I continued with my original protocol in order to only change one variable (learning load) at a time.

Smith's laboratory (C. Smith & Rose, 1997) also described increases in the amount of RS when rats were allowed to sleep normally following training on a spatial learning task. The RS increases did not however overlap with the RD sensitive periods, but instead the increases in RS were delayed by several hours. It seems counterintuitive how two time windows can exist, one that is sensitive to a lack of RS and one that is related to an increase in RS. One would think that if RS is truly important, these two time periods should overlap. Though the results from my studies did not repeat the findings of Smith's group, together the two laboratories do suggest that while still unclear, an interaction between RS and learning load likely exists.

Effects of RS disruption that can affect behavioral outcome

Changes in performance measures associated with RD are not a clear representation of the effects of RD on 'learning', using performance as a correlate of learning. Depending on the task, performance measures can be affected by RD in several ways.

RD can be stressful both due to the physiological effects of RD and as a result of the technique used to induce it. As described throughout this dissertation, the inverted flower-pot technique for RS deprivation (Jouvet, Vimont, & Delorme, 1964) can be a stressful one, with the level of stress affected by the number of pots available to the rats (van Hulzen & Coenen, 1981), the presence of additional rats within the same deprivation chamber, the length of time spent on

the pots, and possibly even the level of water within the chamber (Chapter 3). RD can also be associated with altered metabolism, food intake (e.g. Bhanot, Chhina, Singh, Sachdeva, & Kumar, 1989; Hanlon, Andrzejewski, Harder, Kelley, & Benca, 2005) and percent body weight (e.g. Balestrieri, D'Onofrio, & Giuditta, 1980; Bhanot, et al., 1989; Hanlon, et al., 2005), though these effects are difficult to separate from, and are often measured as signs of altered stress levels.

Aside from stress, RD results in decreased attention (Godoi, Oliveira, & Tufik, 2005), though RD also has been shown to alter the drive for making voluntary movements (Elomaa & Johansson, 1986) and with decreased motivation for seeking food reward (Appendix; Hanlon, et al., 2005). Other studies have also described altered affect following sleep deprivation or disruption (e.g. Bonnet, 1985) and increased sensitivity to pain (for review: Lautenbacher, Kundermann, & Krieg, 2006; Roehrs, Hyde, Blaisdell, Greenwald, & Roth, 2006). Further, humans with sleep disruptions have increased tendencies to take greater risks when making decisions (e.g. Acheson, Richards, & de Wit, 2007; Killgore, Balkin, & Wesensten, 2006). It is difficult to find a task impervious to these side-effects of RD that would focus purely on the impact of RD on learning.

The Appropriateness of Currently Used Tasks

With all of these potential RD-associated factors affecting performance, it is important to be able to separate memory or learning from confounding side-effects of RD on performance. Among the tasks used for analyzing the effects of

REM sleep deprivation or REM sleep restriction on spatial learning and memory are the Morris water maze (Beaulieu & Godbout, 2000; Le Marec, et al., 2001; Li, et al., 2009; Ruskin, et al., 2006; C. Smith & Rose, 1996, 1997; Wang, et al., 2009; Youngblood, et al., 1997), the 8 - arm maze (C. T. Smith, et al., 1998) and the Poe 8 - box maze (Bjorness, et al., 2005).

The Morris water maze is based on finding a hidden platform and escaping from the water, where the search for the platform can be aversive and induce stress. However, the basis of this task depends on equivalent drive amongst groups to desire escape and find the platform. The stress that the Morris water maze task may cause can interfere with sleep (Tang, Liu, Yang, & Sanford, 2005) and with learning (Foy, et al., 1987). In the Morris water maze, a probe trial is typically used to measure memory or retention as an animal can bump into the platform without prior knowledge as to precisely where it is located when the platform is present. While a probe trial is not used in several of the RD and Morris water maze studies (Beaulieu & Godbout, 2000; Le Marec, et al., 2001; Li, et al., 2009; Ruskin, et al., 2006; C. Smith & Rose, 1996, 1997; Youngblood, et al., 1997) it is the most accurate way for attaining an idea of how well the animal remembers the platform location. However, even the probe trial can be contaminated by RD side-effects if there is a severely altered HPA axis or altered drive for escape. It may not be possible to absolutely say whether an animal does not remember the platform location. However, it is possible to confidently say that they do remember the platform location based on the current measures for performance

on probe trials (number of platform crossings, percent time in target quadrant, average proximity to the platform location). In studying the preference for one location over another, as in my study taking the probe trial measurements in reference to both the Learning Phase and Reversal Phase platform locations on Day 6, the effect of RD-associated altered behavior is accounted for.

In my studies, the differences found during Morris water maze training are not profound, and have slightly stronger, though still not large differences on the probe trials. One explanation for this is that in previous studies, it has been shown that the classical form of the Morris water maze can be solved without the use of hippocampal-based learning (Hoh, Beiko, Boon, Weiss, & Cain, 1999). Using different forms of the Morris water maze, reference versus working memory can be measured (Ruskin, et al., 2006; Youngblood, et al., 1997) and compared, making it a stronger task to use, depending on the question. However, even with the comparison of reference versus working memory in the Morris water maze, there is conflicting evidence as to whether RD affects working (Ruskin, et al., 2006) or reference (Youngblood, et al., 1997) memory (see Chapter 1). Overall, the Morris water maze can be a useful assessment tool. Unlike appetitive tasks, the Morris Water Maze allows normal feeding for the experimental animals and it is so often used in learning research that the results are better compared to other learning interventions, even though it may not always provide very strong or convincing results.

The 8 - arm maze is an appetitive reward task, where the rat chooses specific arms in order to attain a food reward. The 8-arm maze does not have a probe trial built into it, however it can be used to discern between spatial working and spatial reference memory. A drawback of using this task is that RD can decrease the motivation to perform a task for a food reward (Appendix; Hanlon, et al., 2005). Further, food deprivation or restriction alters subsequent sleep patterns (Roky, Kapas, Taishi, Fang, & Krueger, 1999), thereby potentially disrupting sleep in control animals, which could mask RD-associated group differences. The ability to differentiate between both working and reference memory on this task makes it useful for comparing how RD affects these two general types of memory when compared to controls. The comparison of these two memory types helps to control for side effects related to the task and to the RD.

The Poe 8 - box maze is designed for rats to run laps of a rectangular maze stopping at specific boxes for a food reward while ignoring all others. This task can be used to differentiate between intramaze and extramaze strategies for knowing the location of the baited boxes on the rectangular maze, with extramaze strategies thought to be dependent on the hippocampus. The 8-box maze is a useful task for comparing how RD affects strategies when compared to controls. However, this task is also vulnerable to motivational effects of RD and to sleep disruptions due to food deprivation or restriction.

With sleep potentially being affected following all of these behavioral learning tasks, home-cage controls may also be undergoing disrupted sleep patterns, obscuring the differences between controls and the experimental group being RD or undergoing rRS. Potentially if both groups have sleep interferences immediately following training, any effect of RS modulation could be lost or masked. All three tasks listed above have the potential for within-task comparisons to be made (e.g. comparing preference for two platform locations, spatial reference versus working memory or intramaze versus extramaze strategies) which can act as a potential controlling factor for the interaction between the task and the sleep manipulation. If a difference is found in one but not the other metric within the same task, then the effects on the task itself are accounted for. Unfortunately, each of these tasks are susceptible to the side-effects of RD, as are many additional ones not mentioned, e.g. fear conditioning (increased sensitivity to pain), bar lever pressing (decreased attention and motivation for food reward). In most cases, with an understanding of how these tasks can be affected by RD-associated side-effects, they can be valuable tools.

Is REM sleep essential for learning?

My data indicate that REM sleep is not always essential for learning. With sufficient training, rats can perform tasks following daily short bouts of RD without any effect on the concurrent spatial task (Chapter 2). I cannot say with full confidence that my results have shown that RS is essential for concurrent learning, as it would be more compelling if there were stronger deficits in

performance following RD (Chapter 4). What is interesting is that future, or subsequent learning is affected by previous RD. With the number of subjects used in my studies it is difficult to elucidate how predictive the performance from the Learning Phase is of performance during the Reversal Phase (Figure 4.17), though my results are statistically significant. Poorer performance on the Learning Phase could enable better learning of the Reversal Phase as it may be easier to 'rewrite' what has not been previously well learned, and vice versa. My data suggest that rRS during the Learning Phase alters the relationship between initial and subsequent performance. Namely, the level of performance a normal sleeping individual had during the initial spatial learning was similar to the level of performance expressed during reversal learning. In contrast, of those rRS during the Learning Phase, poorer performance during the initial spatial learning was associated with better performance during reversal learning.

Instead of being able to soundly put to rest the debate as to the level of dependence of learning on RS, my studies help to tie together some of the existing literature by directly addressing some of their methodological discrepancies. Several studies have previously indicated that RS aids learning (Bjorness, et al., 2005; Fu, et al., 2007; Le Marec, et al., 2001; Li, et al., 2009; Pearlman, 1973; Ruskin, et al., 2006; Silvestri, 2005; C. Smith & Rose, 1996, 1997; C. T. Smith, et al., 1998; Wang, et al., 2009; Youngblood, et al., 1997) while other reputable results have indicated that it is not (for review: McGrath & Cohen, 1978). My studies draw attention to the fact that even within a single

laboratory it is possible to both prove and disprove the importance for RS on learning, depending on the specifics of a protocol and the aspects (e.g. phases and types) of learning addressed. Based on my studies and an overview of previous studies, I would call for more rigorous assessments of performance, for attention to particular details of the deprivation technique and the stages of sleep lost and homeostatic responses elicited while administering RD. A clearer determination of the stress levels induced with various RD techniques and their impact on both sleep and learning itself is required. Further, the question of whether RS is important for learning is a more complex topic than a simple yes / no question, and these important nuances should be strongly considered in future debates.

It would be unreasonable to presume that a clear answer to the level of importance of RS on learning across species is available. As there are so many different types of learning, with a high number of protocol variations frequently used, it would be difficult to determine that RS is important generally for learning. Further, while it may be possible to show that RS is clearly important for a set type of learning, irregardless of widely differing protocols, it would be difficult to draw this conclusion across species, namely because different species value set types of learning over others, and various brain regions are more highly represented or enlarged in some species more than in others. When analyzing recorded sleep / waking states, the length of epoch used (e.g. 10 s versus 30 s) can greatly alter the findings if a ‘majority rules within the epoch’ is used to

determine the epoch's sleep / waking state. With differing techniques for administering RD, the level of RD or deprivation of other sleep states can be greatly altered. Combined, these two factors make it difficult to compare across studies for behavioral results associated with either scored RS amounts or with RD. Lastly, there is an ongoing debate regarding how to characterize RS across species. It is possible that RS evolved at several times throughout evolution. Could it be possible then that for some species RS is relevant for specific types of learning, and for others it is not? An example of this is the comparison between the rat and human where hippocampus dependent learning is thought to be associated with RS in rodents (Bjorness, et al., 2005; Li, et al., 2009; Ruskin, et al., 2006; C. Smith & Rose, 1996, 1997; C. T. Smith, et al., 1998; Wang, et al., 2009; Youngblood, et al., 1997) and non-RS in humans (e.g. Marshall & Born, 2007; Stickgold & Walker, 2005). Therefore, we may not be able to define the role of RS for learning across species.

Future Directions

To be able to support the theoretical model I proposed in Chapter 4, additional experiments are vital. Namely, to temporarily block hippocampal activity for 6 hrs following training on both 12 trials and 4 trials and repeat my studies to determine the effects of blocking consolidation during both the Learning Phase and the Reversal Phase, as well as measuring the effects on subsequent reversal learning. Although it takes considerable time, recording hippocampal cell activity following training in both controls and rRS rats would address if rRS is preventing

cells associated with the Learning Phase platform location from firing at theta troughs at the end of Day 3, and would allow us to identify the firing phase for both the Learning Phase and Reversal Phase platform locations on Days 4 through 6. Further, it would be possible to address whether rRS delays the consolidation window based on the phase of hippocampal cell firing as well. However, unless the recording system for measuring hippocampal cell activity could be waterproofed, an alternative task may be required. A disadvantage of this would be the probable switch to an appetitive-based task, with the previously described potential confounds. Additionally, it is possible that my results may be vulnerable to a change in reward or task. Contrary, it could be an advantage to change to a task that would allow the differentiation between hippocampal and non-hippocampal dependent strategies to solve the task. A possible land-based task, with differentiable strategies, would be the Poe 8-box maze. An alternative future project is the determination if rRS does indeed affect flexibility of learning. To address this the effects of rRS should be tested on a more complex task such as a place response discrimination task, targeted to measure flexibility.

Conclusion

My current studies on the effects of RD on concurrent spatial learning and reversal learning speak to the difficulty of determining the relevance for RD and learning. RD did not have an equivalent effect on two associated learning tasks, initial spatial and reversal learning, when using 4 trials of learning. My current studies also highlight the importance of consistent protocols across studies (level

of water in the deprivation chambers, and potentially subtle details such as richness of room cues). Based on differences across the spatial learning literature, it seems necessary for future reports to provide a detailed account (photograph, video or other) of the extent of cues within the room that subjects can map to. My studies draw attention to the need to reevaluate the previous literature on RS and learning to determine if previous results are affected by either the deprivation technique or overlearning prior to the RS manipulation. The research in this dissertation is the first to describe an interactive relationship between RS and learning load on both concurrent and subsequent learning.

References

Acheson, A., Richards, J. B., & de Wit, H. (2007). Effects of sleep deprivation on impulsive behaviors in men and women. *Physiol Behav, 91*(5), 579-587.

Balestrieri, S., D'Onofrio, G., & Giuditta, A. (1980). Deprivation of paradoxical sleep. Effect on weight and nucleic acid content of liver and brain. *Neurochem Res, 5*(12), 1251-1264.

Beaulieu, I., & Godbout, R. (2000). Spatial learning on the Morris Water Maze Test after a short-term paradoxical sleep deprivation in the rat. *Brain Cogn, 43*(1-3), 27-31.

Bhanot, J. L., Chhina, G. S., Singh, B., Sachdeva, U., & Kumar, V. M. (1989). REM sleep deprivation and food intake. *Indian J Physiol Pharmacol, 33*(3), 139-145.

Bjorness, T. E., Riley, B. T., Tysor, M. K., & Poe, G. R. (2005). REM restriction persistently alters strategy used to solve a spatial task. *Learn Mem, 12*(3), 352-359.

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., & Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J Neurosci, 15*(1 Pt 1), 61-69.

Bonnet, M. H. (1985). Effect of sleep disruption on sleep, performance, and mood. *Sleep, 8*(1), 11-19.

Coenen, A. M., & van Luijtelaar, E. L. (1985). Stress induced by three procedures of deprivation of paradoxical sleep. *Physiol Behav, 35*(4), 501-504.

Conrad, C. D., Galea, L. A., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. *Behav Neurosci, 110*(6), 1321-1334.

Elomaa, E., & Johansson, G. G. (1986). Decision-making to initiate voluntary movements in the rat is altered during deprivation of rapid eye movement sleep. *Neurosci Lett, 63*(1), 51-55.

Foy, M. R., Stanton, M. E., Levine, S., & Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behav Neural Biol, 48*(1), 138-149.

Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., et al. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience, 144*(4), 1186-1192.

Gallagher, M., Burwell, R., & Burchinal, M. (1993). Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci, 107*(4), 618-626.

Godoi, F. R., Oliveira, M. G., & Tufik, S. (2005). Effects of paradoxical sleep deprivation on the performance of rats in a model of visual attention. *Behav Brain Res, 165*(1), 138-145.

Hanlon, E. C., Andrzejewski, M. E., Harder, B. K., Kelley, A. E., & Benca, R. M. (2005). The effect of REM sleep deprivation on motivation for food reward. *Behav Brain Res, 163*(1), 58-69.
Hodges, H. (1996). Maze procedures: the radial-arm and water maze compared. *Brain Res Cogn Brain Res, 3*(3-4), 167-181.
Hoh, T., Beiko, J., Boon, F., Weiss, S., & Cain, D. P. (1999). Complex behavioral strategy and reversal learning in the water maze without NMDA receptor-dependent long-term potentiation. *J Neurosci, 19*(10), RC2.
Jouvet, D., Vimont, P., & Delorme, F. (1964). [Study of Selective Deprivation of the Paradoxal Phase of Sleep in the Cat.]. *J Physiol (Paris), 56*, 381.
Killgore, W. D., Balkin, T. J., & Wesensten, N. J. (2006). Impaired decision making following 49 h of sleep deprivation. *J Sleep Res, 15*(1), 7-13.
Kim, J. J., Shih, J. C., Chen, K., Chen, L., Bao, S., Maren, S., et al. (1997). Selective enhancement of emotional, but not motor, learning in monoamine oxidase A-deficient mice. *Proc Natl Acad Sci U S A, 94*(11), 5929-5933.
Krugers, H. J., Douma, B. R., Andringa, G., Bohus, B., Korf, J., & Luiten, P. G. (1997). Exposure to chronic psychosocial stress and corticosterone in the rat: effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity. *Hippocampus, 7*(4), 427-436.
Lautenbacher, S., Kundermann, B., & Krieg, J. C. (2006). Sleep deprivation and pain perception. *Sleep Med Rev, 10*(5), 357-369.
Le Marec, N., Beaulieu, I., & Godbout, R. (2001). Four hours of paradoxical sleep deprivation impairs alternation performance in a water maze in the rat. *Brain Cogn, 46*(1-2), 195-197.
Li, S., Tian, Y., Ding, Y., Jin, X., Yan, C., & Shen, X. (2009). The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats. *Learn Behav, 37*(3), 246-253.
Marshall, L., & Born, J. (2007). The contribution of sleep to hippocampus-dependent memory consolidation. *Trends Cogn Sci, 11*(10), 442-450.
McGrath, M. J., & Cohen, D. B. (1978). REM sleep facilitation of adaptive waking behavior: a review of the literature. *Psychol Bull, 85*(1), 24-57.
McLay, R. N., Freeman, S. M., & Zadina, J. E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. *Physiol Behav, 63*(5), 933-937.
Pearlman, C. (1973). REM sleep deprivation impairs latent extinction in rats. *Physiol Behav, 11*(2), 233-237.
Peigneux, P., Laureys, S., Fuchs, S., Destrebecqz, A., Collette, F., Delbeuck, X., et al. (2003). Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. *Neuroimage, 20*(1), 125-134.
Poe, G. R., Nitz, D. A., McNaughton, B. L., & Barnes, C. A. (2000). Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. *Brain Res, 855*(1), 176-180.
Ravassard, P., Pachoud, B., Comte, J. C., Mejia-Perez, C., Scote-Blachon, C., Gay, N., et al. (2009). Paradoxical (REM) sleep deprivation causes a large

and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. *Sleep, 32*(2), 227-240.
Roehrs, T., Hyde, M., Blaisdell, B., Greenwald, M., & Roth, T. (2006). Sleep loss and REM sleep loss are hyperalgesic. *Sleep, 29*(2), 145-151.
Roky, R., Kapas, L., Taishi, T. P., Fang, J., & Krueger, J. M. (1999). Food restriction alters the diurnal distribution of sleep in rats. *Physiol Behav, 67*(5), 697-703.
Romcy-Pereira, R., & Pavlides, C. (2004). Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP. *Eur J Neurosci, 20*(12), 3453-3462.
Ruskin, D. N., Dunn, K. E., Billiot, I., Bazan, N. G., & LaHoste, G. J. (2006). Eliminating the adrenal stress response does not affect sleep deprivation-induced acquisition deficits in the water maze. *Life Sci, 78*(24), 2833-2838.
Silvestri, A. J. (2005). REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiol Behav, 84*(3), 343-349.
Smith, C., & Rose, G. M. (1996). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiol Behav, 59*(1), 93-97.
Smith, C., & Rose, G. M. (1997). Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze. *Behav Neurosci, 111*(6), 1197-1204.
Smith, C. T., Conway, J. M., & Rose, G. M. (1998). Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. *Neurobiol Learn Mem, 69*(2), 211-217.
Stickgold, R., & Walker, M. P. (2005). Sleep and memory: the ongoing debate. *Sleep, 28*(10), 1225-1227.
Tang, X., Liu, X., Yang, L., & Sanford, L. D. (2005). Rat strain differences in sleep after acute mild stressors and short-term sleep loss. *Behav Brain Res, 160*(1), 60-71.
van Hulzen, Z. J., & Coenen, A. M. (1981). Paradoxical sleep deprivation and locomotor activity in rats. *Physiol Behav, 27*(4), 741-744.
Wang, G. P., Huang, L. Q., Wu, H. J., Zhang, L., You, Z. D., & Zhao, Z. X. (2009). Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation. *Neuroreport, 20*(13), 1172-1176.
Youngblood, B. D., Zhou, J., Smagin, G. N., Ryan, D. H., & Harris, R. B. (1997). Sleep deprivation by the "flower pot" technique and spatial reference memory. *Physiol Behav, 61*(2), 249-256.

Appendix

The Effects of REM Sleep Restriction on the T-maze

Introduction

Learning

Learning is thought to occur via one of three key centers, the hippocampal complex, the amygdaloid complex and the basal ganglia (for review see White & McDonald, 2002). While the interplay of these three centers may be involved, tasks can be differentially associated with one structure over the other. The hippocampus is associated with spatial tasks, requiring the mapping of the surrounding environment based on distal environmental cues in relation to the individual or subject. The amygdala is associated with tasks requiring associative learning between a reinforcer and a cue(s). An example of this is classical fear conditioning, associating an electric shock with a sound cue. The basal ganglia are associated with tasks that require learning based on linking the cues with the response, and food-reward tasks. An example of this is the T-maze, where the

individual chooses to turn in a specific direction at a choice point (rather than to a specific location) of the maze based on a food-reward. Others have described the basal ganglia as being associated with procedural or implicit or habitual learning as well as motor learning (for review see Pennartz, et al., 2004). Though learning can occur through the interaction of these brain regions, it has also been shown that hippocampal learning can interfere with striatal learning (for review see White & McDonald, 2002). In my current study, I use a T-maze task that cannot be solved using a spatial map, which would involve the hippocampus. Therefore attempts to use a hippocampal-based strategy should only act to impair performance. I employed this task in order to test the hypothesis that REM sleep deprivation following a learning task impacts hippocampal dependent learning and not learning that depends on the basal ganglia. I expected that basal ganglia dependent tasks such as the T-maze would either be benefited or unaffected by interventions that alter hippocampal activity.

Sleep

In the human literature more rapid eye movement sleep (RS) has been correlated with better performance on procedural learning tasks (for review see Hobson & Pace-Schott, 2002; Pennartz, et al., 2004). This said, Pennartz et al. (2004) reported significant reactivation within the basal ganglia following training during slow wave sleep in rats. In the animal literature, REM sleep deprivation (RD) results in performance deficits in spatial learning tasks (Bjorness, Riley, Tysor, & Poe, 2005; Li, et al., 2009; C. Smith & Rose, 1996, 1997; C. T. Smith,

Conway, & Rose, 1998; Wang, et al., 2009). These tasks are predominantly dependent on the hippocampus, which is active during RS. The basal ganglia are also active during RS (Hobson & Pace-Schott, 2002). It was previously theorized that RS was necessary for integrating information rather than for habitual learning (Greenberg & Pearlman, 1974), which suggests that RS would not facilitate our T-maze task. Therefore short bouts of RD or RS restriction (rRS) should have no effect on performance measures in my study.

The original goal of this study had been to determine interactions between the rRS effects on learning with age, using both a hippocampus-based spatial learning task and a basal ganglia-based procedural learning task. However, due to a limited supply in aged animals, I will only report on the effects of rRS on a basal ganglia-dependent learning task irrespective of age.

I hypothesized that rRS would not affect learning on the T-maze, based on previous work within our laboratory (Bjorness dissertation, Chapter 5) and previously posited theories (Greenberg & Pearlman, 1974).

Methods

Animals

All rats used in this study were Fisher 344 male rats aged 13 - 16 months (Middle aged rats) and 27 - 31 months (Older rats) (Harlan Indianapolis, IN). Animals

were housed in a 12:12 light cycle at an average temperature of 23 °C. Procedures were approved by the animal review board, the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. Rats had access to fresh drinking water at all times except about 30 mins / day while on-task. Food was restricted to maintain each rat's body weight at ~ 85 % original body weight prior to testing.

REM sleep deprivation protocol

The REM sleep deprivation protocol used for this study is identical to that described in Chapter 2. Each deprivation chamber had three inverted flowerpots with 2 cm of water at the base of the chamber.

Visual Water Maze protocol

I employed the visual water maze protocol previously described in Chapter 2 to isolate and remove any individual rats with inferior vision or motor ability. Rats that were unsuccessful in reaching the platform after multiple trials, or failed to reach it in a timely fashion (average of less than 30 s) were removed from the study.

Habituation protocol

Prior to T-maze testing, each rat was habituated to the maze and food restricted. This took place across 5 days. Each rat was placed in an arm of the plus-maze

and encouraged to explore all 4 arms of the maze by scattering small pieces of cheerios throughout. Habituation lasted 10 mins per day. The walls of the maze were painted black, and the movable junction wall was red. Each arm floor was lined with a piece of white cloth. At the end of the two goal arms was a small, etched glass bowl, preventing the rat from seeing the Cheerio reward within the bowl. Major room cues surrounded the maze.

Prior to training on the T-maze, rRS rats were well habituated to the deprivation chambers from testing in previous studies.

T-maze

Rats were tested 4 - 6 months after visual maze testing. During this interim, each rat was tested on the hidden platform version of the Morris water maze and tested on an odor recognition protocol. Rats were divided into one of four groups and remained in those groups across all protocols. According to age, rats were divided into a rRS group or a control group. The four groups were: older REM sleep restricted group (OrRS; n = 2), older homecage control group (OCON; n = 2), middle-aged REM sleep restricted group (younger, YrRS; n = 3) and middle-aged homecage control group (younger, YCON; n = 4). As the subject number was low for this pilot study and the differences between older and middle aged animals not clearly evident, I collapsed across age to determine the effects of rRS, resulting in a REM sleep restricted mixed age (rRSM) group and a homecage control mixed age (CONM) group.

Training consisted of fifteen 45 s trials per day for 7 days. Following most trials, the intertrial interval was 1 min long, however after every 5th trial the maze was rotated by 90º and the corresponding intertrial interval was 2 mins. The start arm for each trial was pseudorandomly assigned, with each rat performing trials in the same order. On the first trial on Day 1, both arms contained the reward. Depending on which direction the rat turned on this particular trial, that direction remained the correct choice direction for the goal arm on all future trials across all days for that particular rat.

After habituation, at the start of Day 1, a movable wall was placed at the head of the start arm, creating a T-maze. A Cheerio was placed into a receptacle container at the end of both non-start arms. Depending on which arm the rat chose on this trial, the right or left, this position relative to the start-arm became its target goal for future trials. Therefore, not all rats had the goal of turning left rather than right at the T-junction. If a rat did not leave the start arm within the 45 s allotted for the trial, the trial was noted as an incorrect missed trial and the rat was coaxed into the goal arm. Once a rat had entered it's 2 front paws into either the correct or incorrect goal arm, it was counted as committing to it's chosen arm. Rats were not allowed to reenter a different arm once it had committed to another. If the rat chose the correct goal arm but failed to reach the Cheerio within the allotted time, it was denoted as a correct trial requiring the full trial

length to reach the target. Immediately after training rats in the rRSM group were RS deprived for 4 hrs, while the controls were returned to their homecage.

Statistics

Data were analyzed as the percent correct of the total number of trials per day, the number of correct trials per day, the number of incorrect trials per day, the total number of trials performed per day and latency to food reward. Statistical analyses were performed using repeated measures ANOVA to measure differences across the study, across the first three days of the study and across the last three days of the study. Further, independent *t*-tests were used where appropriate to test for group differences on specific days.

Results

Performance between CONM and rRSM was equivalent at the start of the experiment (Day 1) for correct number of trials run (Figure 1), incorrect number of trials run (Figure 2), total number of trials run (Figure 3), percent correct of total trials run (Figure 4), percent incorrect of total trials run (Figure 5) and latency to reward (Figure 6). However, after the first round of RD, on Day 2, CONM had significantly more correct trials than rRSM ($p = 0.001$, Figure 1), and rRSM had significantly longer latencies to reach the reward as compared to CONM ($p = 0.016$). rRSM remained in the start arm significantly more times than CONM on Day 2 ($p = 0.036$, Figure 3). The number of incorrect trials run (Figure 2),

however, did not differ between the two groups, nor did the percent correct trials run when calculated based on the number of total trials on which the rat left the start arm per day (Figure 4) or the percent number of incorrect trials performed (Figure 5).

I also investigated the effect of rRS across the first three days (Days 1 – 3) and the last three days (Days 5 – 7) of the experiment. During the first part of the experiment, CONM still had significantly more correct trials than rRSM ($p = 0.02$, Figure 1). CONM ran significantly more trials ($p = 0.044$, Figure 3) and tended to have shorter latencies to reaching the reward ($p = 0.087$, Figure 6) than rRSM. When the percent of correct trials performed as a measure of the total number of trials run was anlayzed no significant group difference was identified. Similarly, there was no difference for the number of incorrect trials run or the percent number of incorrect trials performed.

During the latter part of the study, the difference in the number of correct trials performed was no longer significant ($p = 0.056$, Figure 1). rRSM left the start arm on significantly fewer trials than CONM ($p = 0.047$, Figure 3), and CONM still tended to have shorter latencies to the reward ($p = 0.083$, Figure 6) than rRSM. Again, there was no difference between the two groups when the percent of correct or incorrect trials was calculated or the number of incorrect trials performed.

When the entire experiment was considered, rRSM ran significantly fewer trials across the entire experiment ($p = 0.038$, Figure 3), but only trends were seen for CONM to perform more correct trials ($p = 0.051$, Figure 1) with shorter latencies ($p = 0.08$, Figure 6) than rRSM. No group differences were found for the number of incorrect trials or percent incorrect trials performed.

Summary

Deficits in performance were measured starting after the first bout of RD on Day 2. For the first part of the study, rRSM had fewer correct trials than CONM. Throughout the entire study, rRSM left the start arm on fewer trials than CONM. Performance differences were no longer detectable when the number of correct trials was measured against the total number of trials run per day.

Discussion

Unlike my prediction, I found that unless the total number of trials performed per day were accounted for, RS deprived rats were impaired on our procedural T-maze task, performing fewer correct trials. However, throughout the study rRSM left the start arm on significantly fewer trials after the first session of RD. Therefore rRS had no effect on accuracy of performance but instead seemed to impair motivation to perform the task.

Discussion concerning the differing results between my current study and prior work in the lab for the number of correct trials

Previous work in our lab (Bjorness dissertation) found that rRS did not affect the number of correct trials performed on the T-maze task, even though the total number of trials run per day was not presented. It is not clear why my studies produced differing results. Combining the two age groups may have affected the data, with the measurement from the aged rats acting to skew my results. However, looking at a plot of each individual rat's data for the number of correct trials (Figure 7) and the total number of trials performed (Figure 8), it would not appear that the aged rats performed any worse compared to the younger rats.

Exposure to other tasks prior to the T-maze, as well as repeated exposures to the RD chambers may have had an effect on my current results. In Bjorness's dissertation work, the rats used would have had fewer exposures to the RD chamber and other tasks prior to T-maze testing. In my current study, those rats previously RS restricted during other tasks were the same as those RS restricted during this study. Rats that performed the T-maze had previously been trained and tested in the Morris water maze for two 6-day periods and on various forms of an odor recognition task (based on modified version of an odor task used in Dr. Schallert's, personal correspondence 2006). In addition, rats in the current study were tested on a visual form of the Morris water maze. While I do not believe that these tasks should have affected performance on the T-maze, prior exposure to them may have altered brain regional density, propensity to learn

specific strategy types or reliance on previously adapted strategies. Specifically, if prior testing and rRS reversed the predicted results, one would have to conclude that previously RS restricted rats from other experiments were more apt to display learned helplessness for a new task. Additionally, while I would expect prior RD exposures to result in the current RD being less stressful, it is also possible that rats had a negative association with it (e.g. being forced to swim in a water tank had always been followed by RD for the rRS group). At the time, I chose to retain my rats in their original group so that I could compare the rRS effects on spatial and non-spatial tasks. With only 4 hrs of RD per day when in-study, and a minimum of 2 weeks between rRS studies, no persisting increased RS pressure would be expected. To be sure that my rats were not affected from prior exposures to both RD and various behavioral tasks and to further speculate between the differences in my current study and that of Bjorness's dissertation work, I would need to rerun my experiment with naïve rats.

Discussion of the group difference in the number of trials a rat failed to leave the start arm

The increase in the number of trials where the RS deprived rats did not leave the start arm is not altogether surprising. Using an operant conditioning task with a food reward, rats that were RS deprived for 24 hrs a day using the multiple platform method showed decreased motivation for the reward (Hanlon, Andrzejewski, Harder, Kelley, & Benca, 2005). The decrease in motivation was despite the fact that RS deprivation resulted in lower relative body weights and

increased pressure for food intake. Hyperphagia and drop in body weight is a well-documented finding with RD (Dement, 1965), which would predict behavior counter to both my current findings and those previously reported by Hanlon et al. (2005). However, my counterintuitive result also emphasizes the significance of the fact that, irrespective of their increased level of hunger, rats failed to perform the task to attain the food reward.

Moreover, RD has been associated with an increase, rather than a decrease in motor activity (Albert, Cicala, & Siegel, 1970). Previous research has shown that RD does not affect exploration (Boyaner, 1970; Hicks, Okuda, & Thomsen, 1977), therefore rRSM's failure to leave the start arm should not be related to a diminished drive to explore the environment, and if anything increased motor activity could promote an increase in the number of trials to leave the start arm, again emphasizing the significance of the observed decline in completed trials.

Following RD, rats can show signs of heightened stress levels due to an altered hypothalamic-pituitary axis (HPA) response which may be the result of the technique used to administer the RD or the RD itself. I did not measure stress levels in this study (e.g. cortisol, ACTH, adrenal gland weight) and due to my protocol (maintaining body weight at ~ 85% original weight) I cannot postulate stress levels based on changes in percent body weight. The RD chamber which I used allowed rats to move freely between 3 easy-to-reach inverted flowerpots in the presence of a low level of water to reduce the occurrence of the rats' tails

dangling in the water. For longer periods, 6 hrs instead of the 4 hrs employed here, previous work in our lab found that with repeated days of rRS, rats (Chapter 2 and Chapter 4) did not have a significant loss in body weight, indicating that 6 hrs of RD was not more stressful within a 24 hr period than home cage sleeping. To fully address whether stress was a factor in the rRS group failing to leave the start arm, I would need to repeat the study and include measures for stress. It may also be useful to measure the effect of rRS on the T-maze task in a different strain of rats, such as Sprague-Dawleys, to avoid the altered HPA axis seen with the F344 strain.

It could be argued that the RS restricted rats failed to leave the start arm due to tiredness or due to the manifestation of sleep-like states. However, the RD period I used was only 4 hrs in duration which would lead to the necessity for a fairly minor recovery. I would expect this recovery to have been fully completed prior to testing the subsequent day (~ 19 hrs later), since using the same RD chambers and set-up, following much longer RD periods (24 hrs), recovery appears to occur within the first 4 hrs post-RD (Mashour, et al., in review). Further, an experimenter was present throughout the T-maze task and monitored to ensure no animals displayed sleep-like behaviors.

A motivation deficit on the T-maze task has been previously described following a lesion of the nucleus accumbens (Salamone, Cousins, & Bucher, 1994). Improved performance on the T-maze task is associated with higher

acetylcholine (ACh) levels (Chang & Gold, 2003). Therefore it would appear that while choosing the correct arm may be regulated by ACh levels, motivation to leave the start arm is associated with dopamine levels (Salamone, et al., 1994). It is possible to conclude that motivation levels can be affected by RD (Hanlon, et al., 2005). Based on the current literature, it is unclear how dopamine is affected by RD. It appears therefore that the T-maze may be a good task for differentiating between the effects of RD on various neural networks/systems.

My original goal of this study was to determine if age altered the effects of rRS on striatal learning. In a study relating choline acetyltransferase activity (measured according to levels of acetylcholine) with passive-avoidance learning, aging and RS (Stone, Altman, Berman, Caldwell, & Kilbey, 1989), results indicated that aged rats responded similar to young rats with lesioned forebrain cholinergic neurons with a deficit in performance, which correlated with decreases in RS bout length. Additionally, choline acetyltransferase levels were not affected in the hippocampus with age but in both the striatum and the frontal cortex. Based on these findings I would have predicted aged animals to have a deficit in performance measures following rRS. However, my number of subjects was too low to be able to further address this.

Summary

In humans, procedural learning tasks have been closely associated with RS (e.g. Marshall & Born, 2007; Stickgold & Walker, 2005), though this is not supported in

the animal literature. My results describe a lack of rRS-associated deficit in choosing the correct arm (number of correct arms as a percent of total number of trials run) but support a rRS-associated drop in motivation to perform the task (increased number of trials where RS deprived rats failed to leave the start arm). Without increasing my current number of subjects I cannot determine if my present findings are truly common across the lifespan or if they diverge with age.

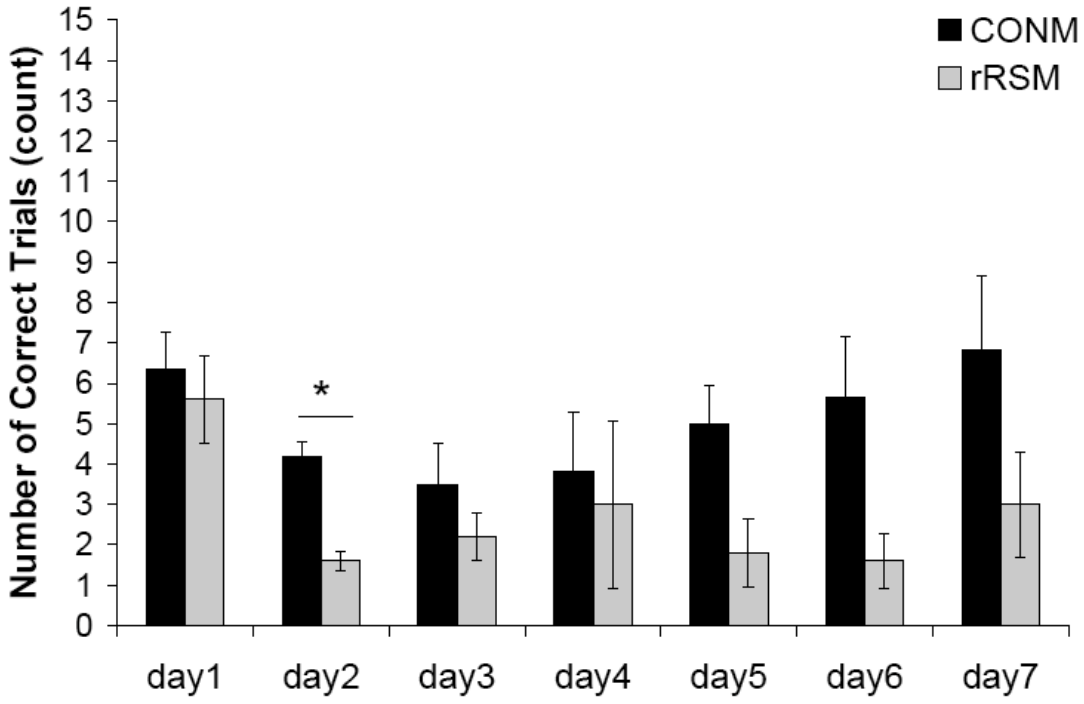


Figure A.1 Total number of correct trials performed on the T-maze
The number of correct trials performed across the 7 days of the experiment are shown as mean ± SEM for CONM (solid black) and rRSM (lined). A total of 15 trials were administered per day.

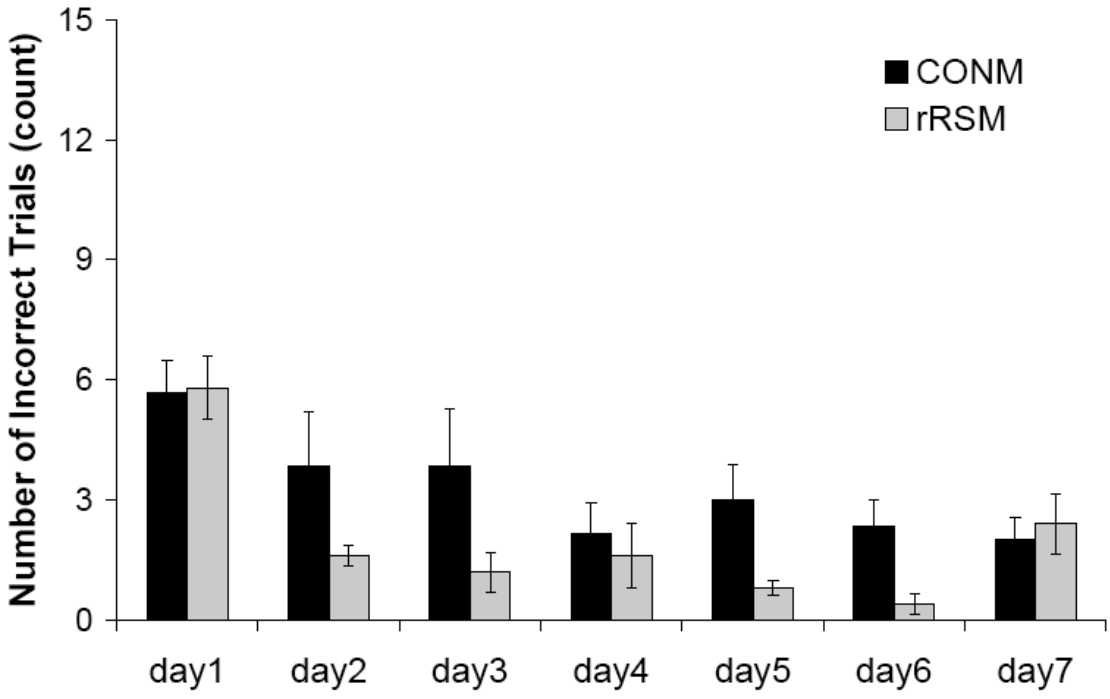


Figure A.2 Total number of incorrect trials performed on the T-maze
The number of incorrect trials performed across the 7 days of the experiment are shown as mean ± SEM for CONM (solid black) and rRSM (lined). A total of 15 trials were administered per day.

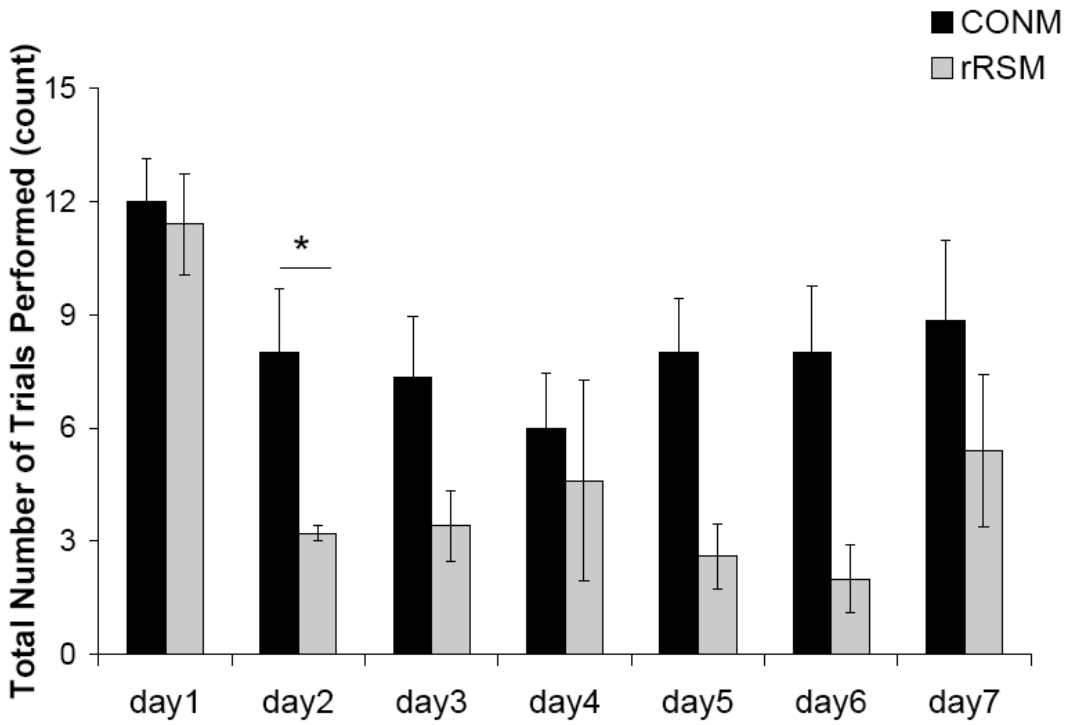


Figure A.3 Total number of trials performed on the T-maze
The total number of trials a rat left the start arm performed across the 7 days of the experiment are shown as mean ± SEM for CONM (solid black) and rRSM (lined). A total of 15 trials were administered per day.

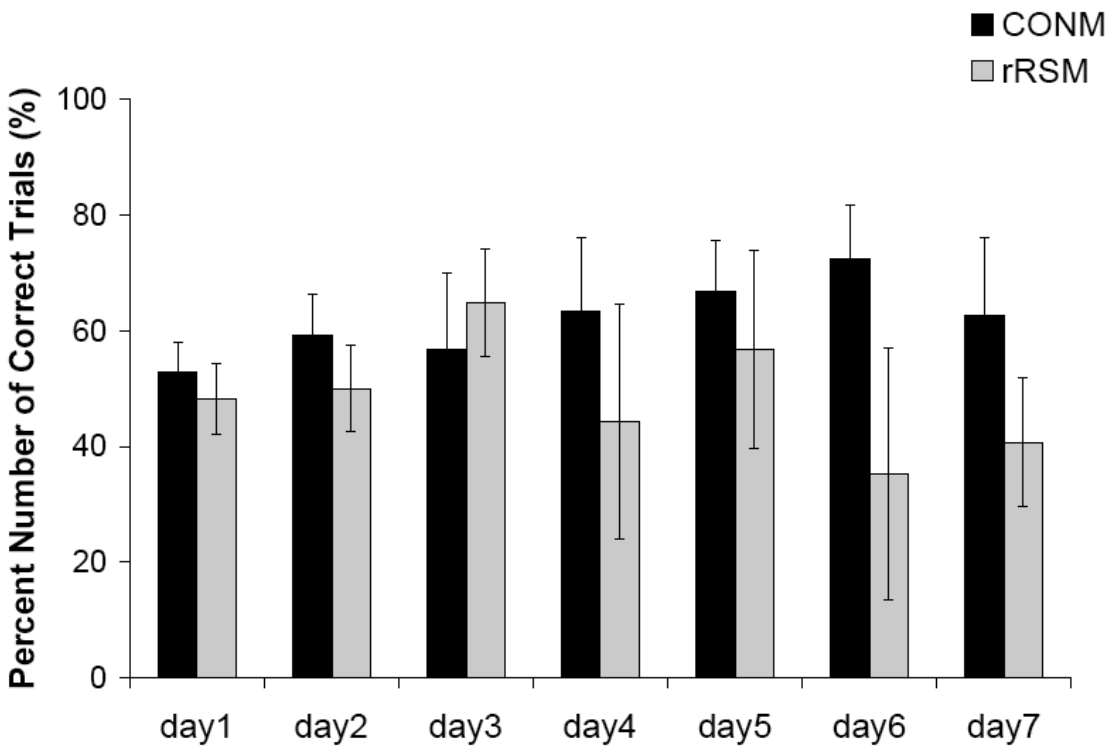


Figure A.4 The number of correct trials as a percent of the total number of trials performed on the T-maze

The number of correct trials as a percent of the total number of trials performed across the 7 days of the experiment are shown as mean ± SEM for CONM (solid black) and rRSM (lined). A total of 15 trials were administered per day.

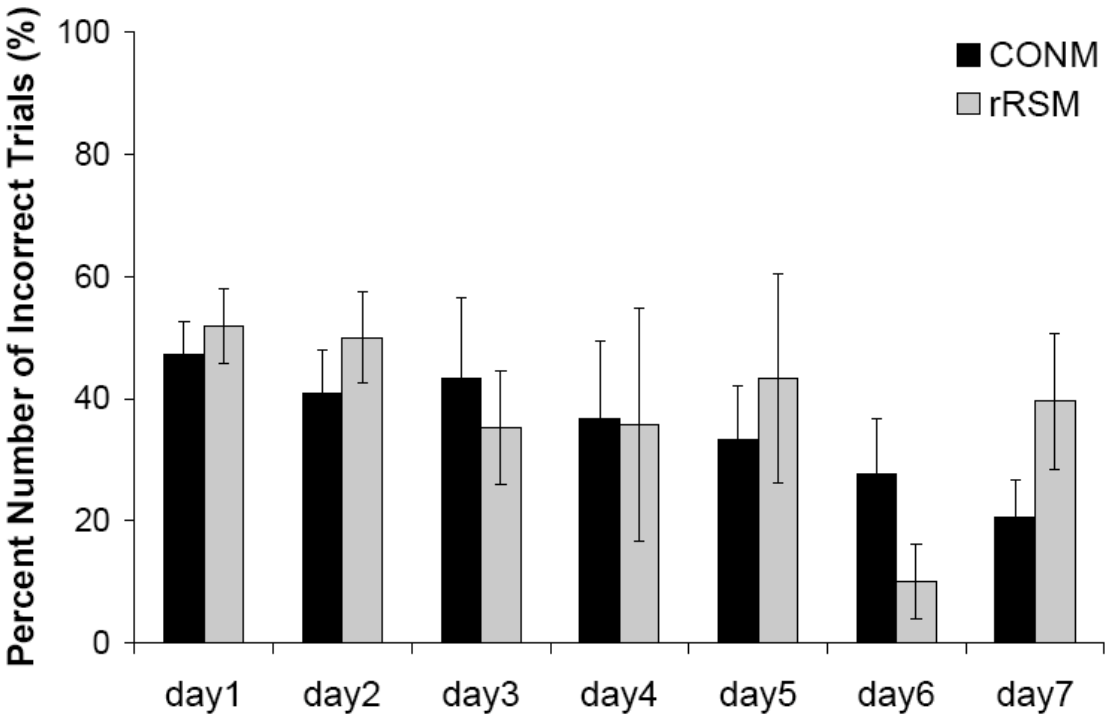


Figure A.5 The number of incorrect trials as a percent of the total number of trials performed on the T-maze

The number of incorrect trials as a percent of the total number of trials performed across the 7 days of the experiment are shown as mean ± SEM for CONM (solid black) and rRSM (lined). A total of 15 trials were administered per day.

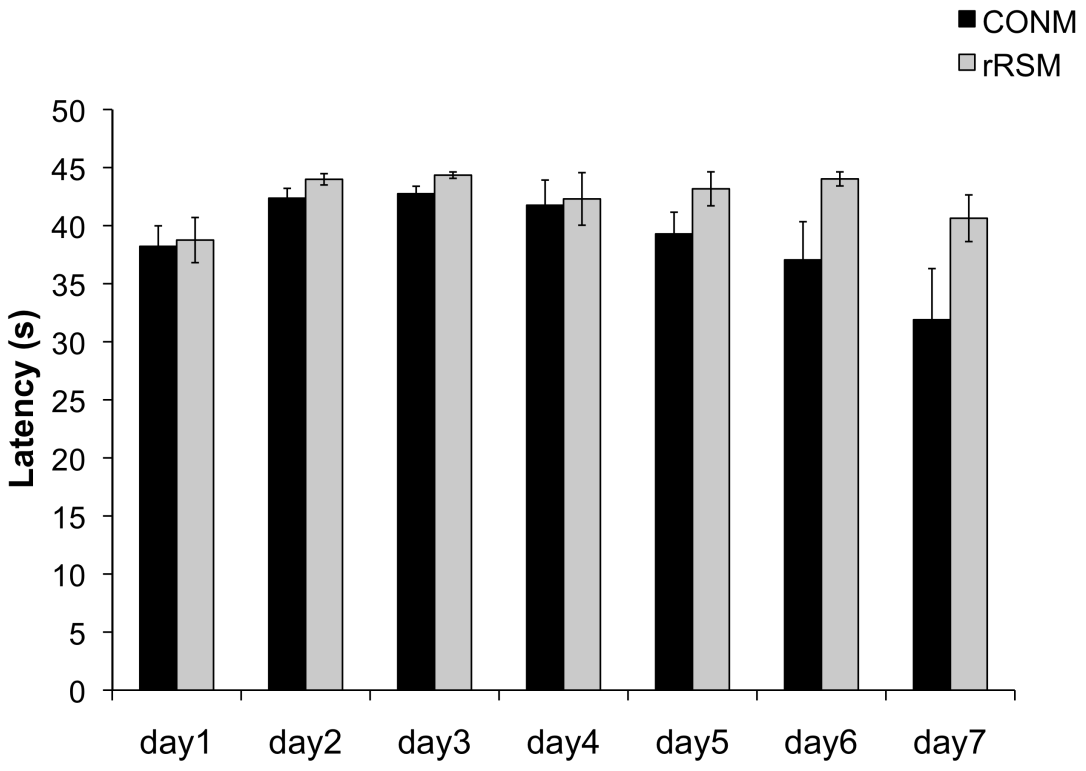


Figure A.6 Trial length
Latency to attain the reward or to commit to the incorrect arm was measured in seconds. If a rat failed to reach the reward within the 45 s trial length it was assigned a latency of 45 s. Data are shown for the 7 days of the experiment mean ± SEM for CONM (solid black) and rRSM (lined). A total of 15 trials were administered per day.

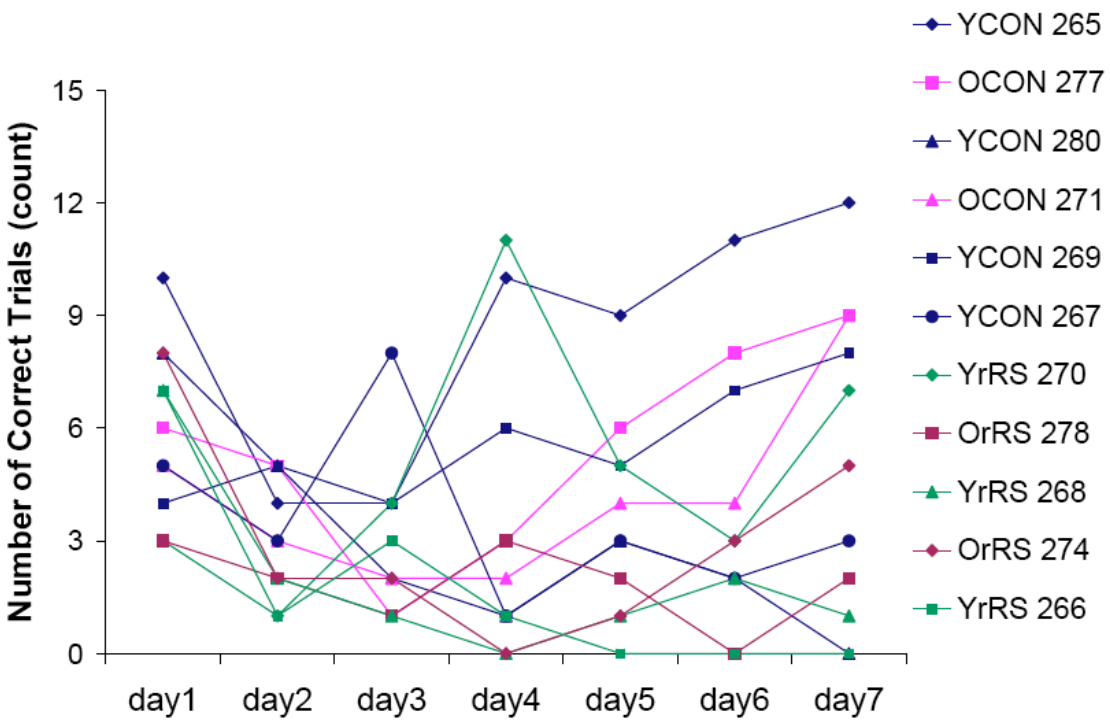


Figure A.7 The number of correct trials performed by each individual rat on the T-maze

The number of correct trials performed across the 7 days by each individual rat is indicated. A total of 15 trials were administered per day. Middle-aged rats (YR, blue), older rats (OR, pink), middle-aged rRS rats (YrRS, green) and older rRS rats (OrRS, purple) along with their assigned rat number is graphed.

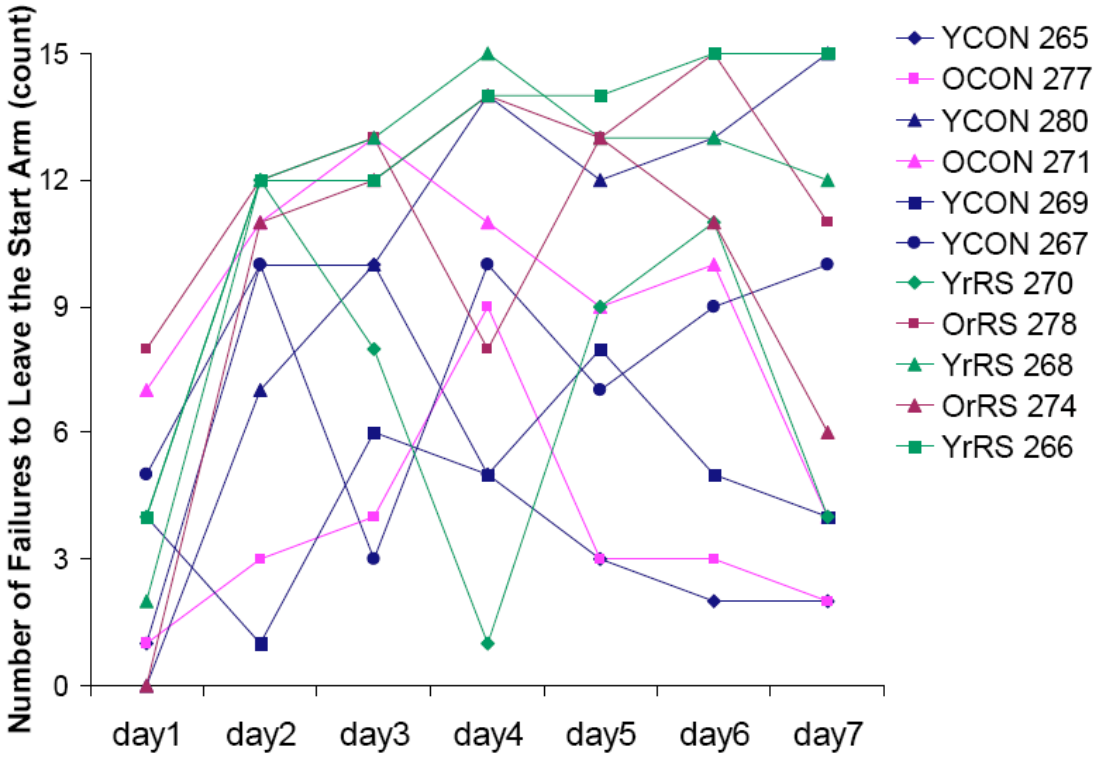


Figure A.8 The total number of trials performed by each individual rat on the T-maze

The total number of trials for a rat to leave the start arm is shown for each individual rat across the 7 days of the experiment. A total of 15 trials were administered per day. Middle-aged rats (YR, blue), older rats (OR, pink), middle-aged rRS rats (YrRS, green) and older rRS rats (OrRS, purple) along with their assigned rat number is graphed.

References

Albert, I., Cicala, G. A., & Siegel, J. (1970). The behavioral effects of REM sleep deprivation in rats. *Psychophysiology, 6*(5), 550-560.

Bjorness, T. E., Riley, B. T., Tysor, M. K., & Poe, G. R. (2005). REM restriction persistently alters strategy used to solve a spatial task. *Learn Mem, 12*(3), 352-359.

Boyaner, H. G. (1970). Effect of REM sleep deprivation on exploration in rats. *Psychol Rep, 27*(3), 918.

Chang, Q., & Gold, P. E. (2003). Switching memory systems during learning: changes in patterns of brain acetylcholine release in the hippocampus and striatum in rats. *J Neurosci, 23*(7), 3001-3005.

Dement, W. C. (1965). Recent studies on the biological role of rapid eye movement sleep. *Am J Psychiatry, 122*(4), 404-408.

Greenberg, R., & Pearlman, C. (1974). Cutting the REM nerve: an approach to the adaptive role of REM sleep. *Perspect Biol Med, 17*(4), 513-521.

Hanlon, E. C., Andrzejewski, M. E., Harder, B. K., Kelley, A. E., & Benca, R. M. (2005). The effect of REM sleep deprivation on motivation for food reward. *Behav Brain Res, 163*(1), 58-69.

Hicks, R. A., Okuda, A., & Thomsen, D. (1977). Depriving rats of REM sleep: the identification of a methodological problem. *Am J Psychol, 90*(1), 95-102.

Hobson, J. A., & Pace-Schott, E. F. (2002). The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nat Rev Neurosci, 3*(9), 679-693.

Li, S., Tian, Y., Ding, Y., Jin, X., Yan, C., & Shen, X. (2009). The effects of rapid eye movement sleep deprivation and recovery on spatial reference memory of young rats. *Learn Behav, 37*(3), 246-253.

Marshall, L., & Born, J. (2007). The contribution of sleep to hippocampus-dependent memory consolidation. *Trends Cogn Sci, 11*(10), 442-450.

Mashour, G. A., Lipinski, W. J., Lee, U., Matlen, L. B., Walker, A. J., Turner, A., et al. (in review). Isoflurane Anesthesia is not Permissive of Homesotatic Processes related to Rapid Eye Movement Sleep.

Pennartz, C. M., Lee, E., Verheul, J., Lipa, P., Barnes, C. A., & McNaughton, B. L. (2004). The ventral striatum in off-line processing: ensemble reactivation during sleep and modulation by hippocampal ripples. *J Neurosci, 24*(29), 6446-6456.

Salamone, J. D., Cousins, M. S., & Bucher, S. (1994). Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behav Brain Res, 65*(2), 221-229.

Smith, C., & Rose, G. M. (1996). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiol Behav, 59*(1), 93-97.

Smith, C., & Rose, G. M. (1997). Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze. *Behav Neurosci, 111*(6), 1197-1204.

Smith, C. T., Conway, J. M., & Rose, G. M. (1998). Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. *Neurobiol Learn Mem, 69*(2), 211-217.
Stickgold, R., & Walker, M. P. (2005). Sleep and memory: the ongoing debate. *Sleep, 28*(10), 1225-1227.
Stone, W. S., Altman, H. J., Berman, R. F., Caldwell, D. F., & Kilbey, M. M. (1989). Association of sleep parameters and memory in intact old rats and young rats with lesions in the nucleus basalis magnocellularis. *Behav Neurosci, 103*(4), 755-764.
Wang, G. P., Huang, L. Q., Wu, H. J., Zhang, L., You, Z. D., & Zhao, Z. X. (2009). Calcineurin contributes to spatial memory impairment induced by rapid eye movement sleep deprivation. *Neuroreport, 20*(13), 1172-1176.
White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiol Learn Mem, 77*(2), 125-184.