IMPACTS OF CHRONIC AND ACUTE PHASE-SHIFTING IN MALE AND FEMALE RATS

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ABSTRACT

This thesis assessed the impacts of acute and chronic phase-shifting on learning and memory in male and female rats. Previous research has revealed impaired retention immediately following circadian disruption and on the acquisition of new associations. However, whether behaviour resumes normality following circadian re-entrainment is unresolved. Following circadian re-entrainment, retention of pre-phase-shift acquired associations on Morris water task (MWT) and a visual discrimination task designed on the 8-arm radial maze were tested. Subsequently, an extradimensional set shift (EDS) using the 8-arm radial maze was performed. Acute circadian disruption negatively impacted retention in males and females, but only male rats without running wheels exhibited impairment following chronic phase-shifting on MWT performance. Retention on the visual discrimination task was impaired following chronic, but not acute, circadian disruption. Chronic, but not acute, phase-shifting negatively impacted performance on the EDS. Generally, phase-shifting produced differential negative impacts on cognitive function in rats.

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LIST OF ABBREVIATIONS

ANOVA – analysis of variance
AVP – arginine vasopressin
BMAL-1 – brain and muscle Arnt-like protein 1
BDNF – brain derived neurotrophic factor
CalB – calbindin
cm – centimeter
CLOCK – circadian locomotor output cycles kaput
CA1 – cornu ammonis 1
CA2 – cornu ammonis 2
CA3 – cornu ammonis 3
CRY – crytochrome
Cry1 – cryptochrome 1
Cry2 – cryptochrome 2
Cry3 – cryptochrome 3
DG – dentate gyrus
DS – dorsal striatum
EC – entorhinal cortex
EDS – extradimensional attentional set shift
GABA – gamma aminobutyric acid
GAD – glutamic acid decarboxylase
GA – glutaraldehyde
GRP – gastrin releasing polypeptide
HPC – hippocampus
LED – light emitting diode
MOFC – medial division of orbital prefrontal cortex
mENK – met-enkaphalin
MWT – Morris water task
NGF – nerve growth factor
Neu-N – neuronal nuclei
OPFC – orbital prefrontal cortex
PACAP – pituitary adenylate cyclase activating peptide
PER – period
Per1 – period 1
Per2 – period 2
Per3 – period 3
PFA – paraformaldehyde
PBS – phosphate buffered saline
PFC – prefrontal cortex
QC – Quebec
R-O – Response-Outcome
5HT – serotonin
S-R – Stimulus-Response
SCN – suprachiasmatic nucleus
VIP – vasoactive intestinal polypeptide
vmPFC – ventral medial prefrontal cortex

CHAPTER ONE: GENERAL INTRODUCTION

Circadian rhythmicity (i.e., the daily oscillation in activity patterns such as sleep and wakefulness) is a ubiquitous phenomenon among living things, ranging from single cell organisms such as algae (Corellou et al., 2009) and cyanobacteria (Yang et al., 2010) to mammals (for review, see Ukai & Ueda, 2010). The fact that circadian rhythmicity is such a phylogeneticly widespread pattern highlights its great importance to life. Indeed, circadian rhythms allow organisms to regulate behaviour in an optimal fashion, thereby facilitating the appropriate enactment of essential biological functions (e.g., resting, feeding). The necessity of circadian rhythmicity to life, in and of itself, provides ample justification for investigating its general underlying principles and properties. From a behavioural neuroscience perspective in particular, studying circadian rhythmicity is of great value because not only does it play a central role in regulating behaviour, but an emerging body of evidence has also implicated abnormal circadian function with several neurological disease states (e.g., Alzheimer's) and cognitive effects (e.g., impaired hippocampal retention; Antoniadis et al., 2000).

The Suprachiasmatic Nucleus

An endogenous circadian pacemaker allows animals to anticipate their active versus inactive periods and prepare physiological functions accordingly. The identification of the endogenous circadian oscillator has proceeded in several distinct phases, spurred by the development of novel methodological techniques. The first studies identifying suprachiasmatic nucleus (SCN) as the site of circadian rhythmicity were

published in the 1970s (Moore & Eichler, 1972; Stephan & Zucker, 1972). The SCN is a self-regulated group of neurons that together create the internal clock residing within the preoptic area of the hypothalamus (see figure 1.1; for review, see Dibner et al., 2010). Although the population of cells differs by species, the SCN contains roughly 20,000 neurons in mammals, constituting this master clock (Antle & Silver, 2005).

Research began with the examination of neural and behavioural activity *in vivo*, and rapidly progressed to chimeric studies showing that transplanted SCN tissue restores circadian function in previously lesioned animals (for review, see Ralph et al., 1993). In fact, not only is function restored, but the observed circadian rhythm is that of the donor.

Electrophysiology and immunolabeling have helped delineate distinct neural regions within the SCN (Antle et al., 2005; for review, see Brown & Piggins, 2007). Several of the genes and proteins that contribute to circadian rhythmicity have also been identified through both careful observation and the use of genetic mutants (Albus et al., 2002; Kilman & Allada, 2009; for review, see Weaver, 1998). Together, these lines of evidence strongly indicate that SCN is the main source of circadian rhythmicity in mammals.

There are a number of challenges to studying SCN function. First, the stability of the oscillatory patterns (i.e., rhythmicity), measured by period variance and onset precision, decreases as one moves from the whole animal to single cell recordings (Butler & Silver, 2009). Consequently, it is difficult to extrapolate what is occurring at the level of the whole organism based on cellular recordings, although correlations can be drawn between *in vivo* and *in vitro* approaches. Second, although the SCN is a relatively small structure, the bi-directionality of the connections makes it difficult to determine where

the SCN fits within this highly dynamic neural network (Krout et al., 2002; Stehle, et al., 2003; Webb et al., 2009). Third, the SCN functions through the incorporation of exogenous cues, making it difficult to determine intrinsic from extrinsic modulatory effects.

The SCN is comprised of at least two populations of phenotypically distinct cells that are traditionally broken down into two regions: the core and the shell (Lee et al., 2003). The ability of the SCN to respond to environmental input is modulated by several distinct neurotransmitter systems and patterns of activation. In general, cells comprising the shell portion of SCN are positive for arginine vasopressin (AVP). The shell synthesizes several neuroactive substances including GABA, calbindin (CalB), AVP, angiotensin II, and met-enkephalin (mENK). The core typically exhibits cells positive for vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP). The core also synthesizes GABA, CalB, VIP, calretinin, GRP, and neurotensin (Abrahamson & Moore, 2001). Neurons expressing VIP and GRP (i.e., the core) receive afferent inputs from the downstream structures (such as the retina & regions of hypothalamus and thalamus) and use this input to synchronize the individual oscillators (Stehle et al., 2003). Optic nerve stimulation triggers glutamate and pituitary adenylate cyclase-activating peptide (PACAP) in the SCN, showing how environmental stimuli can lead to excitation within the SCN (Beaule, et al., 2009).

GABA is the major inhibitory transmitter in the adult mammalian central nervous system (Keck & White, 2009) and is detectible in virtually every neuron in the SCN and associated structures (e.g., the retina, intergeniculate leaflet of the lateral geniculate nucleus; Harrington, 1997). It has been argued that GABA is the source of variability in

several domains because variable inhibition can mediate the level of excitatory input to or from a neuron (Moore, 1993). A peak in GABA expression is observed within the SCN at the midpoint of the active period in mammalian species (Friedman & Piepho, 1978). Pharmacological studies provide further evidence for the role of GABA in the SCN by showing that manipulating levels of GABA, precursors such as glutamic acid decarboxylase (GAD), or receptors leads to changes in both behavioural rhythms and cellular activity within the SCN (Castel & Morris, 2000; Shirikawa et al., 2000).

In addition to the role of GABA, SCN function is partially modulated by serotonin (5HT). The process by which 5HT, and closely related melatonin, impact circadian rhythmicity is not yet completely understood although it is proposed that 5HT inhibits photic phase-shifts by inhibiting the release of glutamate, thereby decreasing the responsiveness of retinorecipient cells (Sterniczuk et al., 2008). Several distinct 5HT receptor subtypes have been identified within the SCN, and all appear to be component processes of the circadian system (Smith et al., 2008).

Individual cells within the SCN express rhythmicity, driven by autoregulatory transcription-translation feedback loops (for review, see Yan, 2009). The individual rhythms are coordinated daily via external input that regulates the individual rhythms to produce a coherent output (Antle & Silver, 2005). The feedback loops are dependent on two transcriptional activators, CLOCK and BMAL1. These proteins heterodimerize and bind to E-box elements in the promoters of target genes. This triggers two transcriptional repressors, PERIOD (PER; *Per1, Per2,* and *Per3*) and CRYPTOCHROME (CRY; *Cry1, Cry2,* and *Cry3*) proteins, among others (for review, see Fukada & Okano, 2002). When PER and CRY proteins accumulate in the cytoplasm, they enter the nucleus and inhibit

their own expression by repressing CLOCK/BMAL-1 mediated transcription (Chen et al., 2009). It takes approximately 24 hours for the PER and CRY proteins to reach critical levels of each stage of the feedback loop to repress their expression (Tournier et al., 2003). The activation and repression of these factors is the source of the daily intracellular oscillations observed in SCN.

The intracellular endogenous rhythms *in vivo* are independent of surrounding cells. Even if disrupted for several days, the endogenous oscillatory pattern will reemerge (Welsh et al., 1995). Although cells found within the SCN maintain rhythms independent of environmental stimuli, exogenous input is necessary for proper function. Exogenous cues align the population of individualized oscillators, ensuring that they cycle in a cohesive fashion (Welsh et al., 2010).

In the natural environment, exogenous cues such as light and temperature have consistent rhythms. Hence, these cues are reliable and provide information that the neural system can rely upon to entrain the oscillators. In unnatural environments, such as modern cities or the laboratory, however, this reliance can be manipulated. Consequently, inconsistent cues produce arrhythmicity within the SCN. Although the impacts of manipulating this schedule are well documented in SCN, the impacts on other neural regions are still not fully understood.

Phase-shifting

Salient environmental stimuli that comprise the exogenous cues influencing circadian rhythm (i.e., *Zeitgebers*), and the adjustments they elicit in the circadian system under purposive manipulation is called a *phase-shift*. Manipulating zeitgebers (e.g., introducing an artificial sunrise in the middle of the night) provides a method for

disturbing circadian function that does not rely on altering neural regions directly. Noninvasive disruption of circadian rhythms is typically accomplished by altering light schedules, which is effective because the SCN is heavily reliant on retinal input (Morin & Allen, 2006). In rats, phase-shifts are typically induced by adjustment of the light schedule (Illnerova & Sumova, 1997). Alternatively, phase-shifting can also be induced through the use of restricted and enforced eating, drinking, and exercise schedules. It is thought, however, that these cues act primarily on mechanisms outside the CNS and, therefore, influence the SCN in a less direct fashion (Dallmann & Mrosovsky, 2006; Girotti, et al., 2009; Hirota & Fukada, 2004).

In addition to being a model for shift-work or jetlag (Reddy et al., 2002; Selgado-Delgado, et al., 2008), phase-shifting is a method of inducing circadian disruption that is typically observed in association with several disease states. The impairments elicited by phase-shifts resemble the circadian rhythm abnormalities observed with aging, Alzheimer's disease (for review, see Van Someren, 2000), or cancer (Blask et al., 2005). Other relationships exist in terms of normative brain function and daily circadian rhythms including, but not limited to, the increased incidence of stroke in early morning (Lewis et al., 2010), altered hormone profiles that contribute to a vast array of behaviours (Few et al., 1987), and time of day effects on cognitive ability (Allen et al., 2008; Bennett et al., 2008). Furthermore, the intensity of events such as stroke or seizure activity appear to be partially mediated by current circadian state (Sanabria et al., 1996; Tischkau et al, 2007).

The effects of phase-shifting within the SCN are well documented (Chen et al., 2008; Molyneux et al., 2008; Smith et al., 2010) and include altered gene expression, desynchronization between the core and shell regions, and resetting of the clock to the

adjusted time. Phase-shifting targets the SCN by triggering the release of serotonin (for review, see Challet, 2007). Although the endogenous oscillator and the zeitgebers that act upon it are interesting in and of themselves, how circadian desynchonization impacts higher order functions such as learning and memory has yet to be fully developed. Although not examining the impacts of phase-shifting directly, the study of sleep and sleep deprivation often reveal cognitive impairments (for review, see Walker, 2008). Sleep deprivation could be contributing to any observed effects, but it is likely not the only source of cognitive impairments (Sil'kis, 2009; Tartar et al., 2010; Lee et al., 2009).

Memory Systems

Hippocampus

The hippocampus (HPC) was first described over 400 years ago. Research conducted during recent decades has begun to unravel the contributions made by the HPC toward learning and memory processes. Early animal studies examining hippocampal morphology and function failed to isolate this structure, both functionally and physiologically from other areas, such as amygdala, striatum, or over and underlying cortical regions (for review, see Crinella, 1993). The imprecision of these lesions lead to several erroneous assumptions regarding hippocampal contributions to learning and memory (for review, see Izquiredo & Medina, 1998).

In the 1950s a patient (H.M.) with severe temporal lobe epilepsy underwent nearly complete bilateral removal of his hippocampus. Removal of H.M.'s hippocampi led to a profound loss in his ability to learn new information. Through careful experimental design, researchers were able to ascertain that the observed impairments

were found in a contextual, or autobiographical domain. Conversely, H.M.'s ability to perform learning tasks that were formed during habit or motor learning, such as the mirror drawing task, was left intact (Milner, 2003; Scoville & Milner, 1957). The finding that some aspects of learning and memory can be lost while others remain intact benefited the whole of neuroscience by providing a conceptual framework for understanding the organization of learning and memory in the mammalian brain.

The behavioural impacts observed following H.M.'s surgery have been replicated in animal models. In rats, hippocampal lesions produce impairments during some, but not all, learning paradigms (McDonald et al., 2004). In rats, the hippocampus is critical for incorporating information from several sensory modalities and combining these disparate cues into a cohesive representation of the context of a particular experience (Schmajuk & Blair, 1993). There are several behavioural assays that have been developed to assess hippocampal function, including Morris water task (Sutherland et al., 1982), contextual conditioning (for review, see Fanselow, 2000; Sutherland & McDonald 1990), and configural/pattern completion tasks (Leutgeb & Leutgeb, 2007; Rudy & Sutherland, 1989). Episodic memory has also been exhibited in rodent models using single pairings of contexts and shocks in a fear conditioning to context paradigm (Hunsaker et al., 2008). As is the case with H.M., stimulus-response (S-R) learning is largely unaffected by hippocampal lesions in rats (McDonald et al., 2004; McDonald et al., 2007a). In fact, research has shown that certain types of learning (i.e., S-R learning; McDonald et al., 2004) are enhanced by eliminating HPC, lending support to the parallel processing theories (White & McDonald, 2002). The profound functional importance of the

hippocampus is partially responsible for the extensive and continued study of this structure.

Like the many functions attributed to the HPC, the methods by which this structure is examined vary widely. Investigative approaches include permanent (i.e., electrolytic lesions; Maren & Fanselow, 1997) or temporary inactivation of the HPC (i.e., muscimol injection; Maren & Holt, 2000). In addition to lesions directly to the HPC, cells projecting to and from it have been damaged to see how the HPC operates within the system (Ferbinteanu et al., 1999; Sutherland & Rodriguez, 1989).

Another interesting feature of the HPC is that it is very sensitive to many environmental perturbations such as periods of stress (Narayanan & Chattarji, 2010), hypoxia/ischemia (Pereira et al., 2009), circadian disruption (Craig & McDonald, 2008; Graves et al., 2003), a combination thereof (McDonald et al., 2008a), and others. It has been proposed that the reason that the HPC is so susceptible to damage is that, by necessity, it is a highly malleable (i.e., plastic) structure (McEwen & Milner, 2007). For example, learning is associated with observable changes in HPC, especially at the level of the synapse (Briones et al., 2005; Leuner & Gould, 2010).

Hippocampal Morphology

The HPC is a heterogeneous structure comprised of several regions that can be clearly delineated by morphological, pharmacological, and functional differences (for review, see Witter, 2007; see figure 1.2). These interconnected areas are traditionally defined as entorhinal cortex (EC), subiculum, dentate gyrus, cornu ammonis 1 (CA1), cornu ammonis 2 (CA2), and cornu ammonis 3 (CA3). Each of these regions serves a distinct purpose within the hippocampal operational pathway.

Electrophysiology has been used extensively in the examination of HPC (for review, see Mockett & Hulme, 2008). In fact, recent developments in recording techniques have allowed for recording from HPC and other neural structures simultaneously (Lansink et al., 2007). Electrophysiological recording has revealed distinct cell types, including place cells, grid cells, head-directional cells, and episodic or sequence cells, with different functions attributed to each. Place cells, located within the CA1 and CA3 regions of the hippocampus, only fire when the rat is at a particular location in a given context and are thus thought to be involved in learning spatial cues by encoding a certain point in space relevant to other locations (for reviews, see Barry & Burgess, 2007; Jeffery, 2007). Grid cells, located in the EC, operate in association with place cells. Similar to the recordings generated in place cells, grid cells fire at specific locations in space (Moser et al., 2008; Moser & Moser, 2008). The pattern of activation exhibited by grid cells appears to be less flexible than that observed from place cells (Moser et al., 2008; Moser & Moser, 2008). In addition to place and grid cells, the hippocampus also contains head directional cells. As the name suggests, head directional cells, located in the subiculum, fire when the animal is facing a particular direction, regardless of where the rat may be in a navigational field (for review, see Taube & Bassett, 2003).

From an information processing perspective, the first step within the hippocampal system is entorhinal cortex (EC). The EC can be conceptualized as the bottleneck through which information must be filtered prior to hippocampal activation (Furtak et al., 2007). Cell types within the EC vary according to the cortical layer in which they are found, although most layers contain pyramidal cells and various GABAergic interneurons (Jones

& Woodhall, 2005). The EC, which is defined as a polysensory area, receives input from frontal, parietal, temporal, and occipital cortices (Canto et al., 2008). The EC also receives inputs from subcortical structures such as the amygdaloid complex and several nuclei within the striatum, thalamus, and hypothalamus (Zhang & Bertram, 2002). In turn, the EC sends this information to the rest of the hippocampal formation through a series of pathways. Cells from layer II of EC project to the DG and CA3 while cells in layer III project to CA1 and subiculum (Amaral & Witter, 1989). Lesion studies have revealed that, in rats, when EC is damaged but the rest of the hippocampus remains intact, rats show impairment during fear conditioning and extinction (Ferbinteanu et al., 1999; Ji & Maren, 2008; Lewis & Gould, 2007).

The dentate gyrus (DG), receiving its major inputs from EC through the perforant path (Witter, 2007), is commonly held as the second step in the hippocampal pathway. The DG also receives some input from septal and subicular structures (Cavdar et al., 2001). The principle cell type in the DG is the glutamatergic granule cell, although there are also GABAergic interneurons. However, only granule cells send projections to other hippocampal regions, specifically CA3, via the mossy fiber pathway (for review, see Miki et al., 2005). It has been reported that damage to dentate gyrus results in difficulties encoding spatial cues (Gilbert et al., 2001; Xavier & Costa, 2009) such as impairment during performance of the Morris water task (Rudy & Sutherland, 1989) or object-context mismatch tasks (Spanswick & Sutherland, 2010).

Cornu Ammonis 3 (CA3) is one of the three regions comprising Ammon's horn (i.e., the hippocampus proper; Witter et al., 2000). Pyramidal cells of CA3 tend, on average, to be larger than pyramidal cells in CA1. Cornu Ammonis 2 (CA2) is very

similar to CA3 and the distinguishing feature between CA2 and CA3 is that CA3 alone receives projections from DG (for review, see Witter et al., 2000). In addition to the input CA3 receives from DG and EC, it also receives cholinergic input from the medial septal nucleus and the nucleus of the diagonal band of Broca (Witter, 2000). Although it receives substantial inputs from other regions, CA3 is largely interconnected, creating a massive association network within it (for review, see Miki et al., 2005). In turn, CA2 and CA3 project to CA1 via the Schaffer Collaterals (Witter et al., 2000). Lesion studies have shown that CA3 is important during the performance of spatial tasks such as MWT (Okada & Okaichi, 2010).

In addition to inputs via the Schaffer Collateral pathway, Cornu Ammonis 1 (CA1) also receives projections from EC. Cells within CA1 tend to be smaller and spaced further apart than their counterparts in CA3 or CA2. CA1 is the first hippocampal region to project back to EC. In addition, considerable connectivity is present between CA1 and the subiculum (for review, see Witter, 2007). Lesion studies have revealed that damage to CA1 results in impairments in the ability to process temporal cues during pattern separation (Gilbert et al., 2001).

The subiculum, which can be further broken down into the presubiculum and parasubiculum, is the final structure included in the hippocampal formation (for review, see Witter, 2007). The subiculum is the major efferent structure of the hippocampal formation (for review, see Witter, 2007). The subiculum has considerable connectivity with cortical (e.g. perirhinal, entorhinal) and subcortical (e.g. diencephalons, brainstem; for review, see Witter et al., 2000) regions. It also possesses substantial inputs from several thalamic and hypothalamic nuclei (e.g. paraventricular nucleus, amygdaloid

complex; Cooper et al., 2006). Interestingly, lesions of subiculum have revealed the role played by other structures (i.e., amygdala, prefrontal cortex) that are largely hidden when HPC is intact (McDonald & White, 2002). In addition to the lesion studies, single unit recording in this area has lead to the discovery of head directional cells that are thought to be important for successful navigation (Muller et al., 1987).

In summary, the hippocampus is part of a neural system important for complex learning and memory functions and is highly sensitive to various types of perturbations. In addition, most disease states during which circadian disruption is observed also include characteristic dysfunction of the HPC. Therefore, the examination of changes in HPC, such as behavioural performance or synaptic morphology, following circadian disruption should reveal interesting effects.

Prefrontal Cortex

Like the hippocampus, the functional importance of PFC was revealed by an unusual clinical case. This case involved a man named Phineas Gage, and the railroad construction accident that resulted in a spike being driven through his PFC. Initially, no behavioural deficits were observed following his accident, but problems with rationality and emotional regulation soon emerged (for review, see Damasio et al., 1994).

Various executive functions have been attributed to prefrontal cortex (PFC) including emotional regulation, problem solving, predictive ability, contingency tracking, and others (Bell et al., 2010; Milad et al., 2007; Mushiake et al., 2009; Raine et al., 2006). Although smaller than that of humans, PFC has been observed in other mammalian species including monkeys and rodents (for review, see Kolb, 1984; Seamans et al., 2008). The PFC is comprised of several distinct regions with different functions

attributed to each (Kolb, 1984), although not always in the same location or proportion across species.

In rats, the PFC is characterized by extensive input from the dorsal thalamus and is comprised of several distinct subregions that fulfill distinct functions (Levy & Goldman-Rakic, 2000; see figure 1.3). The medial network of the orbital prefrontal cortex (OPFC) includes parts of ventral medial prefrontal cortex (vmPFC) and anterior cingulate (AC), as well as parts of the medial division of the OFC (mOFC). Many believe that OPFC is important for the expression of behavioural flexibility due to extensive patterns of connectivity with amygdala, lateral hypothalamus, periaqueductal gray, and hippocampus (for review, see Gabbott et al., 2005).

Several additional functions are also attributed to the OPFC including anticipatory behaviours, maintenance of behavioural and emotional response patterns, strategic planning, and contingency evaluation in order to maximize reward or minimize punishment (Milad & Roesch, 2007; Ostlund & Balleine, 2007). Perhaps of more importance is the emerging body of literature espousing OPFC as a modulator of downstream structures producing behavioural inhibition. For example, animals with lesions to OPFC are impaired during performance of certain types of extinction learning (Zelinski et al., 2010). Specifically, during performance of this fear conditioning to context paradigm, rats with lesions to OPFC show elevated and generalized fear responses and do not extinguish associations at the same rate as controls, indicating a failure to update the original association with the new information.

The PFC is comprised of large pyramidal cells and various interneurons regulated by glutamate and GABA (for review, see Elston, 2003). In addition, the PFC receives

extensive dopaminergic input from the ventral tegmental area (VTA), and dopamine depletion leads to behavioural impairments similar to those associated with lesions to PFC (for review, see Seamans & Yang, 2004). Although dopamine is an important neurotransmitter for normal functioning in the PFC, other neurotransmitters are also important (for review, see Robbins & Roberts, 2007).

Interestingly, normal PFC function is altered following circadian dysfunction, be it phase-shifting or the effects of SCN lesions (Perez-Cruz et al., 2009). Specifically, cognitive functions attributed to PFC appear to be affected by circadian dysfunction in humans (Couyoumdjian et al., in press). This should be alarming given that many medical professionals work schedules that induce arrhythmic circadian profiles (Frank & Ovens, 2002). In addition, differences in PFC have been observed by sex (Kolb & Stewart, 1988). Although it is not critical for all cognitive functions, OPFC does play a role in refining execution. It is likely that impairments incurred during manipulations like phase-shifting would fail to produce gross impairment in PFC, but subtle impacts could be occurring below most detection thresholds.

Striatum

Like the HPC, the striatum is a subcortical structure important for learning and memory. The striatum is characterized by extensive efferent connectivity from cortical sensory and motor areas, the thalamus and the substantia nigra. Major outputs of the dorsal striatum are the thalamus and PFC but also include motor structures (e.g., globus pallidus; White & McDonald, 2002). In addition, the dorsal striatum maintains connections with the hippocampal processing pathway via the entorhinal cortex and subiculum (McDonald et al., 2004).

Traditionally, the striatum is divided into the dorsomedial and dorsolateral striatum (see figure 1.4). The dorsolateral striatum has been implicated in the acquisition of stimulus-response (S-R) associations (Featherstone & McDonald, 2004), whereas the dorsomedial striatum has been implicated in the expression of S-R associations (Featherstone & McDonald, 2005; McDonald & Hong, 2004). S-R associations require the subject to learn the relationship between a particular stimulus and a given response, which leads to reinforcement. Eventually, this type of responding becomes automatic and is thought to lead to habit-like behavioural patterns. In fact, this structure, in conjunction with the extensive patterns of connectivity with amygdala, has been implicated with diseases such as Obsessive-Compulsive Disorder (OCD) and addiction (for review, see van den Heuvel et al., 2010). This type of learning is typically not as sensitive to perturbations.

Mitochondria

It is likely the case that phase-shifting would not produce gross morphological effects like necrosis or apoptosis. Therefore examination of plastic mechanisms in malleable neural regions was conducted. During periods of metabolic stress, the cell must compensate for increasing demands by boosting energy production (Chan et al., 2009). Major sites of energy production in the cell are mitochondria and consequently, there is a relationship between the mitochondrial density of a cell and the metabolic demands it faces (Skulachev, 1999). Metabolism is not the only source of variability in mitochondrial density/function, sex differences in mitochondrial density have also been reported (Irwin et al., 2008). In addition, mitochondrial density is affected by periods of ischemia and hypoxia (Zhan, et al., 2002; Gutsaeva et al., 2008) and aging (LaManna, et

al., 2004). In 2003, Hardeland and colleagues reported that circadian disruption leads to increases in oxidative stress. If metabolically stressful events lead to an increase in the production of mitochondria, and phase-shifting is metabolically stressful, it makes sense to predict that phase-shifting should lead to a change in the number of mitochondria.

Circadian Impacts on Higher Brain Function

The SCN possesses extensive connections with neural regions in a bidirectional fashion. This input/output system allows environmental inputs to regulate the system. It also allows manipulations of the SCN (i.e., phase-shifts) to impact the other structures. Changes elicited in the SCN have already been noted following phase-shifting (Antle & Silver, 2005), so examination of connected regions involved in the performance of behavioural tasks following periods of circadian disruption was conducted. Disruption of a healthy circadian rhythm produces cognitive impairments in non-human and human animals (Devan et al., 2001; Eckel-Mahan & Storm, 2009). These cognitive impairments can also be observed as the result of extrinsic manipulation of the circadian rhythm (i.e., jetlag or shift-work; Reddy et al., 2002; Selgado-Delgado, et al., 2008) or in association with advanced age or disease states that are also characterized by circadian arrhythmicity. (Antoniadis et al., 2000; Benca et al., 2009; Blask et al., 2005; Bombois et al., 2010; Mendlewicz, 2009; Pyter et al., 2010).

Previous research directly related to the research in the present thesis examined the effects of variable lengths of circadian disruption on learning and memory in rats. This research examined how phase-shifting impacts the *acquisition* of hippocampal associations (Craig & McDonald, 2008; Devan et al., 2001) and showed that significant functional impairments were present (Craig & McDonald, 2008; Devan et al., 2001).

The present studies were designed to investigate the impact of acute and chronic phase-shifting on the *retention* of previously acquired associations in both male and female rats. Analyses of mitochondrial density was conducted in conjunction with Morris Water Task (MWT) as a measure of hippocampal function, and a visual discrimination task using the 8-arm radial maze to assess dorso-lateral striatum and PFC function. Furthermore, rats were tested following circadian rhythm re-entrainment, further delineating this from previous work.

CHAPTER TWO

EXPERIMENT ONE: ACUTE PHASE-SHIFT

The impacts of environmental stimuli on intrinsic circadian pacemakers are well documented (for review, see Weaver, 1998). Manipulations of this system have resulted in evidence that phase-shifting is associated with metabolic (Butler & Silver, 2009), physiological (Duguay & Cermakian, 2009), and behavioural changes in several neural regions (Antoniadis et al., 2000) including hippocampus (Craig and McDonald, 2008) and prefrontal cortex (Perez-Cruz, et al., 2009).

The HPC is critical for several cognitive processes including spatial navigation and cells in the HPC are vulnerable to environmental stressors (McDonald & White, 1993; Smith & Mizumori, 2006; Sutherland & Hamilton, 2004; Sutherland, et al., 1982; White & McDonald, 2002). Previous work has revealed learning impairments on MWT following acute and chronic phase-shifting (Craig & McDonald, 2008; Devan et al., 2000). However, several questions about circadian effects on learning and memory persist.

In order to assay HPC function, rats were trained to locate a hidden platform on the MWT, acutely phase-shifted, and examined for retention of the platform location. In addition, the question of how acute circadian disruption alters the function of other cognitive systems, (i.e., dorsal striatum or PFC), was also investigated. Furthermore, whether these effects persist beyond circadian re-entrainment (i.e., normalization of daily running wheel activity), was investigated. Sex was also examined, as some phenomena

appear to produce differential effects in males and females (e.g., stress; Shors et al., 2001). Previous research has determined that circadian disruption impacts memory acquisition, retention and consolidation (Craig & McDonald, 2008; Devan et al., 2001). Therefore, whether any observed effects are emergent from memory retention, retrieval, or both was assessed. Histological quantification was conducted using mitochondrial density.

Brain Areas of Interest

The present experiment assessed hippocampal retention using the Morris water task (MWT). Successful performance of the MWT requires the ability to incorporate various cues from the environment to create a map that can be used to locate a platform hidden beneath the surface of the water. Rats with permanent (Maren & Fanselow, 1997) or temporary (Maren & Holt, 2000) inactivation of hippocampus exhibit impairment during performance of the MWT (Schenk & Morris, 1985; Sutherland et al., 1982). Acute circadian disruption has been associated with impairment during the performance of this task when both training and testing occur following phase-shifting (Craig & McDonald, 2008). However, previous research examined the impacts of acute circadian disruption on the acquisition of hippocampal dependent memories whereas the present research assessed the retention of a previously acquired association.

Assessment of the function of the dorsal striatum was conducted using a visual discrimination variant of the 8-arm radial maze. In the classic version of this task, a food reward is placed at the end of arms delineated by the presence of some cue (e.g., a light). Once the animal has entered the arm and received the reward, the light is extinguished. This means the rat is not responsible for remembering which arms have previously been

entered as much as learning to turn into arms that are light and avoid darkened arms. The version of the task employed in the present experiment included textured floor panels in half of the reinforced arms. As it was not possible to remove the panels during testing, the lights were also left on. Therefore, the rats needed to monitor which arms they had visited. The inclusion of the two cue types was so a second training phase dependent on PFC could be included.

PFC is involved in decision-making and exhibits interesting changes in association with sleep, or sleep deprivation in rats (Montes-Rodriguez et al., 2009) and humans (Couyoumdjian, et al., 2009). In fact, alterations to dendritic morphology have been noted in PFC after episodes of learning (Kolb & Whishaw, 1998). Traditionally, the performance of extradimensional attentional set shifts (EDS), a measure of behavioural flexibility, is dependent on PFC (Ragozzino, 2007). Following acute phase-shifting, retention of the original association was assessed and then asymptotic performance levels were reestablished. At this point, the reinforcement contingencies during performance of the 8-arm radial maze were reversed. That is, during original training rats were trained with four lit arms, two of which included panels. The EDS phase of training involved switching these reinforcement contingencies to four panels, two of which included lights. In order to successfully perform an EDS the subject must be able to track the reinforcement contingencies of each stimulus as well as identify, process, and incorporate alterations in reinforcement contingency.

Potential mechanisms of brain dysfunction

Exogenous events can lead to alterations in the density of mitochondria. In fact, changes have been observed following ischemia/stroke (Zhan et al., 2002), hormonal

manipulation (Irwin et al., 2008), and hypoxia (Gutsaeva et al., 2008). Alterations in mitochondrial morphology are also observed in association with aging (for review, see LaManna, et al., 2004) and altered hormone expression (Irwin et al., 2008). There is a relationship between many of these factors and circadian disruption (Dickmeis, 2009; Skulachev, 1999). Therefore, mitochondrial density was examined following acute circadian disruption.

Accordingly, Experiment One assessed the effects of acute phase-shifting on different cognitive systems (hippocampal, frontal, and dorsal striatum) in male and females rats. Several questions were pursued including: (1) Does acute phase-shifting impair retention on tasks assaying HPC or dorsal striatum function? (2) Does acute phase-shifting impact behavioural flexibility critically dependent on PFC? (3) Does acute phase-shifting impact males and females in a similar fashion? (4) Is mitochondrial function the source of any observed effects?

Methods

Subjects. Twenty female and twenty male Long-Evans rats obtained from Charles River, QC were used in this study. Rats were randomly assigned to six groups (phase-shifted females, phase-shifted males, non-shifted females and non-shifted males, no wheel control females, and no wheel control males). One female rat for the phase-shift condition was excluded from analyses as were both no wheel control groups due to an imbalanced experimental design. The remaining four groups were each comprised of seven subjects. Rats were individually housed in Plexiglas cages equipped with a 42.5 cm

diameter running wheel in a temperature-controlled room. The 12 non-phase-shifted rats housed in standard cages were excluded from analyses because the equivalent groups of phase-shifted rats were not included. All animal handling procedures were performed in accordance with Canadian Council on Animal Care and the University of Lethbridge Animal Care Committee policies.

Phase-shift. (Table 2.1) After a period of acclimation to the solitary running-wheel equipped cages, the light schedule in the housing room was adjusted. In order to elicit a phase-shift, daily light on and offset was advanced by 3 hours for a period of six days followed by a circadian re-entrainment period lasting approximately 3 weeks. Daily activity rhythms were recorded using *Clocklab* software.

Behavioural Tasks

Rapid Acquisition Morris Water Task

Apparatus. A circular pool with a diameter of 1.4 meters and a depth of 40 cm, filled to 70% capacity with cool water, approximately 21 degrees Celsius, rendered opaque using non-toxic acrylic paint was used for this task. The clear Plexiglas platform was concealed approximately 2-3 cm below the surface of the water.

Data Collection. Data were collected using a computer rat tracking system (*VP118, HVS Image*). An over-pool camera recorded all activity within the maze from the time the rat was placed in the arena until it located the platform or 60 seconds had elapsed.

Training. Rats experienced six training sessions over six days during which the invisible platform was located in the East quadrant of the pool. Rats had one session per

day comprised of 8 trials that lasted a maximum of 60 seconds. The rat was left on the platform for approximately 10 seconds before being removed from the maze. If the rat failed to locate the platform by 60 seconds, the experimenter placed the animal onto the platform and left it there for 10 seconds. The subject's path-length and the latency (in seconds) to the platform were analyzed.

Retention Test. After circadian re-entrainment rats were tested for retention of the location of the platform. During a single probe trial the rat was placed into the pool in the south quadrant. There was no platform in the pool and the rat's ability to locate the platform and to search other quadrants when it became aware that the platform was not located in the original location was evaluated.

Visual Discrimination Task Using the 8-Arm Radial Maze

Apparatus. The apparatus used was an eight arm radial maze. The maze is comprised of a central platform with eight arms radiating out. The maze is a wood structure that has been painted white. The floor of the maze arms was covered with a light panel that is smooth on one side and rough on the other. A food cup was located at the end of each arm along with an LED light bar.

Training

Acquisition. Prior to the phase-shifting procedures, animals were trained for 34 days on the stimulus-response (S-R) variant of the eight-arm radial maze. During training four of the arms were delineated by a small light at the end of the arm. Two of these four arms also contained a textured floor panel. Lit and textured arms were reinforced with a food reward located in a food cup at the distal end of each reinforced arm. The purpose behind balancing the reinforcement contingencies of the two cues was to manipulate the

degree to which the cue predicted the reward and to evaluate how these contingencies alter the behaviour of the animal. During this phase of training, lights (100% predictive) were twice as predictive of reward when compared to floor panels (50% predictive) (see figure 4.1a). Once the rats acquired asymptotic performance levels (approximately 80% success rate), this phase of training was complete.

Retention/Retraining. Following phase-shifting and circadian re-entrainment procedures, subjects were tested for retention of the original association. Following retention testing, subjects had two additional days of training to re-acquire asymptotic performance levels.

Extra-dimensional Set Shift. Following retention testing and re-training, the reinforcement contingencies of the light and texture stimuli were reversed. Floor panels were now present in four arms with two of the four also being distinguishable by lights (see figure 4.1b). Animals were trained under this condition for a period of 10 days, until asymptotic performance was achieved under this condition. The purpose behind reversing this cue contingency was to assess the behavioural flexibility, or the ability to switch strategies.

Histology

Electron Microscopy

Rats were deeply anaesthetized using sodium pentobarbital (120 mg/kg) and transcardially perfused with approximately 150 mL of phosphate buffered saline (PBS) followed by a 4% paraformaldehyde (PFA)/2.5% glutaraldehyde (GA) solution. The brains were sectioned at a thickness of 350 microns on a vibrating microtome. Tissue

from the dorsal HPC (dentate gyrus, CA1, & CA3), and OPFC was dissected out. The tissue blocks were washed in .1 M cacodylate, post-fixed in 2% osmium tetroxide/1.5% potassium ferrocyanide in .1 M cacodylate buffer for 2 hr and then stained with 2% uranyl acetate for 45 minutes. Following staining, the tissue samples went through a series of alcohol dehydration steps followed by propylene oxide and gradual embedding in Eponate resin and mounted in a resin block. The blocks were trimmed and two silver sections were cut using a diamond knife and an ultramicrotome. Sections were mounted on a copper grid and stained with 2% uranyl acetate and 7% lead citrate. These procedures are identical to those reported by Kleim, Lussnig, Schwarz, Comery, and Greenough (1996). Specimens were then analyzed using a *Hitachi H7500* Transmission Electron Microscope at 3 kx magnification. The usable area of the specimens was assessed with *ImageJ*, whereby any occluded area was subtracted from the total area to achieve a net area. The mean remaining area for all samples was 98%, with scores ranging from 82 to 100%. Mitochondrial density was assessed using *StereoInvestigator.*

Results

Circadian Rhythms

Phase-shifting and reentrainment. Daily running wheel activity was recorded over the course of the study. During the six days prior to phase-shifting, rats began running shortly after light offset (overall number of minutes between light offset and activity onset, M = 53.14, SD = 75.88), with univariate analysis of variance (ANOVA) showing no main effects or interactions of sex and phase-shift. For a post-phase-shift measure, the

average activity onset for the six days immediately following the phase-shift cycle was calculated. A univariate two way ANOVA (sex X phase shift) showed significant differences between phase-shifted (M= -167.15, SD = 60.60) and non-phase-shifted rats (M = 68.27, SD = 39.54), $F(1,18) = 107.12$, $p < .001$. To ensure rhythm stabilization had occurred prior to retention testing, the average activity onset for the six days immediately before retention testing was calculated. A univariate two way ANOVA (sex X phase shift) showed significant differences between phase-shifted (M= -124.09, SD = 30.38) and non-phase-shifted rats (M = 86.77, SD = 54.08), $F(1,18) = 115.51$, $p < .001$. Although there was a difference in the average onset, a repeated measures ANOVA, with degrees of freedom corrected using Greenhouse-Geisser due to sphericity violation, over the six days comprising this measure showed no interaction of day and phase shift, $F(1.167, 22.182) = .396$, $p = .567$. The lack of an interaction effect indicates that rhythm stabilization had occurred for both the phase-shifted and the non-phase-shifted groups, despite the differential activity onset.

Exercise Wheel Activity. Comparison of the activity profiles was conducted to determine whether running wheel activity patterns differed by sex of phase-shift. A repeated measures ANOVA with equal variances not assumed (Mauchly's $p < .001$) did not reveal significant differences in activity by day $F(2.174,34.779) = 1.527$, $p = .231$. No significant interactions were observed between activity profile and sex $F(2.174,34.779) = 1.319$, $p = .282$, activity profile and phase shift $F(2.174,34.779) = 1.193$, $p = .318$, or all three (i.e., activity profile, sex, and phase-shift) $F(2.174,34.779) = 1.067$, $p = .360$.

Behavioural Tasks

Morris Water Task

Acquisition

Trial duration. The results of the hidden platform location training are depicted in Figure 2.1 a. As can be seen, all of the groups learned the location of the hidden platform. A four-way repeated measures ANOVA (sex X phase-shift X day X trial) performed on trial duration (seconds) over the course of acquisition training showed that all groups learned the location of the platform in a similar fashion. Although main effects of day and trial were observed (*ps.* < .001), no main effects or interactions were observed for any of the between-subject factors (i.e. sex and phase-shift).

Pathlength. Figure 2.1b shows the rate at which all the groups acquired the MWT as measured by path-length to find the platform. A four-way repeated measures ANOVA (sex X phase-shift X day X trial) performed on path-length (cm) during acquisition training showed a similar pattern of behaviour. Again, significant effects were only noted for day and trial (*ps.* < .001) and no significant main effects or interactions were observed for any of the between-subject factors.

Retention (Probe)

Data for the probe trial were grouped into four separate bins labeled: bin one (full trial), bin two (0-10 seconds), bin three (10-20 seconds), and bin four (20-30 seconds).

Bin one (full probe). A repeated measures ANOVA assessing retention of the platform location as indicated by the percentage of time spent in the target versus the average percentage of time spent in the other quadrants revealed a significant interaction

between quadrant and phase-shift, $F(1,21) = 14.599$, $p = .001$. Post hoc, independent samples *t*-tests revealed that phase-shifted rats (M = .24, SD = .14) spent significantly more time in the other quadrants than the non-phase-shifted rats (M = .19 SD = .05), $t(23) = 3.621$, $p = .001$. Furthermore, non-phase-shifted rats (M = .46, SD = .14) spent significantly more time in the target quadrant than did the phase-shifted rats (M = .29, SD = .08). Figure 2.2a represents the performance of the phase-shifted and non-phase-shifted animals during bin one.

Bin two (0-10 seconds). A repeated measures ANOVA assessing retention of the platform location as indicated by the percentages of time spent in the target versus the average time spent in the other quadrants revealed a significant interactions of quadrant and sex, $F(1,21) = 5.173$, $p = .034$, and quadrant and phase-shift, $F(1,21) = 15.067$, $p = .001$. Figure 2.2b and 2.2c represent the performance of males and females and phase-shifted and non-phase-shifted rats during bin two, respectively. Post hoc, independent samples *t*-tests revealed that phase-shifted rats (M = .22, SD = .06) spent significantly more time in the other quadrant than did the non-phase-shifted rats (M = .11, SD = .09), $t(23) = 3.638$, $p = .002$, and that non-phase-shifted rats (M=.66, SD = .27) spent significantly more time in the target quadrant than those that were phase-shifted (M = .33, SD = .17), $t(23) = -3.578$, $p = .002$. The source of the interaction between quadrant and sex is most likely due to the females' relatively greater preference for the target relative to other quadrant compared to males (see figure 2.3b).

Bin three (10-20 seconds). A repeated measures ANOVA assessing retention of the platform location as indicated by the percentages of time spent in the target versus the

average time spent in the other quadrants did not reveal any significant main effects or interactions for any of the variables.

Bin four (20-30 seconds). A repeated measures ANOVA assessing retention of the platform location as indicated by the percentages of time spent in the target versus the average time spent in the other quadrants revealed no significant effects or interactions for any of the variables.

Visual Discrimination Task Using the 8-arm Radial Maze

Acquisition. The results for the acquisition of the visual discrimination task developed for the radial arm maze are depicted in Figure 2.3 a-c. As can be seen, all of the groups learned to enter lit arms for reinforcement and avoid non-reinforced unlit arms as measured by choice accuracy (Figure 2.3a), errors (Figure 2.3b), and re-entries (Figure 2.3c). In order to analyze acquisition, days were grouped into blocks. Two-way repeated measures ANOVAs indicated that all groups acquired the task in a similar fashion in terms of choice accuracy, percentage of erroneous entries (i.e., entering dark arms), or percentage of reentries. No significant main effects or interactions were observed for any of the factors.

Retention. Figure 2.4a-c shows the differences in retention of the visual discrimination across the groups. It appeared that the males showed better retention of the discrimination than did females. A two-way univariate ANOVA performed for choice accuracy on the first retention day (sex X phase-shift) revealed a significant effect of sex, $F(1, 22) = 5.533$, $p = .028$. Independent samples *t*-tests revealed that males (M = .91, SD = .10), performed significantly better than females (M = .73, SD = .23) on the first

retention day, $t(16.54) = 2.449$, $p = .026$. Two-way univariate ANOVAs for errors and for reentries on the first retention day did not reveal any main effects or interactions. Results indicated that male rats exhibit better retention than females.

Retention. Figure 2.5 indicates that groups did not perform differently across the final test day and first retention day. Three two-way repeated measures ANOVAs comparing choice accuracy, percentage of errors, and percentage of reentries on the final training day to the first retention day (sex X phase-shift) did not reveal any significant main effects or interactions.

Re-training. Figure 2.6a-c indicates that over the course of retraining the groups did not differ. To assess group differences in retraining three two-way repeated measures ANOVAs (sex X phase-shift) of overall performance across retention testing for choice accuracy, percentage of errors, and percentage of reentries were conducted. These analyses did not reveal any significant main effects or interactions.

Extra-dimensional set shift. To ensure that the rats were responding to the switch in cue, the first selection on the final training day was compared to the first selection on the final EDS training day using a repeated measures ANOVA. No significant main effect of cue type was observed ($p = .332$), nor were there any interactions with sex ($p = .332$) or phase-shift ($p = .695$). Figure 2.7a-c, representing the performance of each group over EDS training, shows that none of the groups were significantly different. Three two way ANOVAs (sex X phase-shift) of choice accuracy, percentage of errors, and percentage of reentries following the extra-dimensional set shift (EDS) across days revealed no significant main effects or interactions. These results indicated that the groups did not differ to a significant degree in switching strategies.

Three two way ANOVAs (sex X phase-shift) of choice accuracy, percentage of errors, and percentage of reentries following the extra-dimensional set shift (EDS) across blocks revealed no significant main effects or interactions. These results indicated that the groups did not differ to a significant degree in switching strategies.

Histology

Electron Microscopy

Prefrontal Cortex. Figure 2.8a shows the average density of mitochondria in the OPFC. A univariate ANOVA (sex X phase-shift) for the number of mitochondria in OPFC revealed no significant main effects or interactions (see figure 2.9 for an example photomicrograph).

Dorsal Hippocampus. Figure 2.8b shows the density of mitochondria in the HPC. A univariate ANOVA (sex X phase-shift) for the number of mitochondria present in the HPC revealed no significant main effects or interactions (see figure 2.10 for an example photomicrograph).

Discussion

Results indicate that phase-shifting produces impairments on MWT platform location retention in male and female rats. A sex difference was also noted for MWT platform retention, whereby females appear to show better platform location retention than males. Sex differences were also observed during retention testing on the visual discrimination task, although males outperform females on this measure. Extra-

dimensional set shift testing failed to reveal any significant differences between these groups on any measures (choice accuracy, percentage of errors, and percentage of perseverative errors). Histological analyses also failed to reveal any significant differences across groups for the number of mitochondria in OPFC or HPC. Together, these results indicate that the behavioural impairments during the performance of hippocampal-dependent tasks following acute circadian disruption persists beyond circadian entrainment, although mitochondrially mediated metabolic function is not likely the source.

Morris Water Task. All groups acquired the MWT in a similar fashion. Analyses of the percentage of time spent in the correct quadrant versus the average spent in the other quadrants revealed that, over the 30 second trial, non-phase-shifted rats exhibited better retention than phase-shifted rats. During the first 10 seconds of the probe, the non-shifted females spent more time searching the target quadrant than all other groups.

Visual Discrimination Task for the 8-arm Radial Maze. The standard 8-arm radial maze is dependent on dorsal striatum (DS). The task employed here differed from standard S-R radial maze paradigms by the inclusion of the extra-dimensional set shift following retention testing. The reinforcement contingencies were switched following the reinstatement of asymptotic performance. The ability to track such changes in contingency is thought to be dependent on OPFC in rats. These findings suggest that the OPFC was not affected indefinitely by circadian disruption, if at all. Although no significant differences were noted for phase-shift, males exhibited better retention of the S-R association than females.

Electron Microscopy. Mitochondrial density in HPC and OPFC may not be affected by acute phase-shifting. It is possible that the number or function of mitochondria in these regions is only transiently affected by circadian disruption. This could be tested by assessing behavioural function and mitochondrial density immediately after phase-shifting or at various time points within the phase-shifting schedule.

Implications of Results

There are several implications associated with the observed findings. First, even a relatively short period of circadian disruption can impact hippocampal retention, even after circadian rhythm normalization. This finding is novel and warrants further investigation both on the underlying mechanisms and in other animal models. A decrease in hippocampal retention could have serious consequences in humans as many individuals who experience periods of circadian disruption work in emergency services where mistakes are potentially fatal (for review, see Frank & Ovens, 2002).

Superior performance of males on variants of the 8-arm radial maze have been reported elsewhere (for review, see Jonasson, 2005). It is possible that males are relying more heavily on this system than females, which accounts for the superior performance on radial maze and inferior performance on MWT exhibited by males. However, if this was the case, males should exhibit an impairment in the flexibility of the association and this not the case. Therefore, the finding that males exhibit superior performance on the 8-arm radial maze is likely due to superior retention.

Future Directions and Limitations

In the future, time point analyses of all variables should be conducted because it could be the case that physiological changes are transient. Stated simply, by the time

brains were collected for histological assessment, compensation or normalization may have already occurred. Mitochondrial density could be only transiently impacted by circadian disruption. In other words, by allowing circadian rhythm re-entrainment to occur, any change in mitochondria may have also returned to baseline. It is also possible that neural impacts may reside in other domains such as dendritic morphology (Bell et al., 2009), cell function (Reddy et al., 2005), neurotransmitter efficacy (Mohawk et al., 2007; Nakagawa & Okumura, 2010), receptors (Bova et al., 1998), or plasticity (Butler & Silver, 2009). Stereological investigation could reveal some interesting effects, particularly if focused on cell death, birth, and survival across the phase-shift cycle.

Conclusion

Acute circadian disruption not only interferes with the ability to encode hippocampal dependent memories (Craig & McDonald, 2008; Devan et al., 2001), it impacts the retrieval of previously acquired associations. Furthermore, the superior performance of females during MWT retention is novel and warrants further investigation. Impairment following acute circadian disruption is meaningful in and of itself, but the persistence of impairments after circadian rhythm reentrainment should alert industries employing rotating shift work schedules to review scheduling practices and revise accordingly.

CHAPTER THREE

EXPERIMENT TWO: CHRONIC PHASE-SHIFT

Introduction

Results from Experiment One indicate that acute disruptions to normal circadian function produce long-term impairments in the function of the HPC but not PFC or dorsal striatum (see Chapter Two). Therefore, the primary directive of Experiment Two was to ascertain whether extending the period of circadian disruption would induce impairments in the function and integrity of the HPC and produce impairment in the function of dorsal striatum and/or PFC. All procedures were identical to those employed in Experiment One with the exception of the inclusion of running wheel as a between subjects factor in the analyses. Running wheel was included as a factor to ensure that observed effects were related to the phase-shifts and not an effect of exercise.

Previous research examining the impact of chronic circadian disruption (i.e., phase-shifting consisting of repeating the acute phase-shift schedule used in chapter two, four times) showed that this extended form of circadian dysfunction produces impairments in hippocampal function (Craig & McDonald, 2008). Although the paradigm used to elicit a phase-shift was the same, the present research examined the long-term impact of chronic phase-shifting on the *retention* of previously acquired associations dependent on HPC, dorsal striatum, and PFC whereas Craig and McDonald (2008) examined the impact of phase-shifting on the acquisition and retention of hippocampal dependent memories alone.

This experiment investigated the following questions: (1) Does chronic phase-shifting have long-term effects on the brain that impairs retention on tasks assaying HPC or dorsal striatum function? (2) Does chronic phase-shifting have long-term effects on the brain that impact PFC dependent behavioural flexibility? (3) Does chronic phase-shifting have long-term effects on males and females in a similar fashion? (4) Is mitochondrial function the source of any observed effects? (5) Does running wheel activity mediate any effects of phase-shifting or produce any effects independently?

Methods

Subjects. Twenty-two female and 22 male Long-Evans rats obtained from Charles River, QC were used in this study. Animals were randomly assigned to eight groups. Four groups of seven subjects were individually housed in Plexiglas cages equipped with a 42.5 cm diameter running wheel in a temperature-controlled room. In addition, four groups of four subjects were individually housed in standard Plexiglas tubs without running wheels. Three animals were excluded from analyses due to illness or injury. Excluded subjects included one male rat from the phase-shifted no wheel group, one male rat from the phase-shifted with wheel group, and one female from the phase-shifted with wheel group. All animal handling and procedures were performed in accordance with the Canadian Council on Animal Care and the University of Lethbridge Animal Care Committee policies.

Phase-shift. (Table 3.1). After a period of acclimation to the new cages and isolated living, the light schedule was adjusted. In order to elicit a phase-shift, the

following schedule was repeated four times for a total of 64 days. The daily light on and offset was advanced every day by three hours for six days followed by a 10-day partial re-entrainment period before the cycle was repeated. Daily activity rhythms were recorded using *Clocklab* software.

Behavioural Tasks

Rapid Acquisition Morris Water Task

Apparatus & Data Collection. All procedures were identical to those used in Experiment One.

Training & Retention Testing. Rats were trained for a total of seven days. All procedures were identical to those used in Experiment One.

Visual Discrimination Task: 8-Arm Radial Maze

Apparatus. The apparatus used for this experiment was the same as that used in Experiment One.

Training

Acquisition. Prior to phase-shift, rats were trained for 29 days on a visual discrimination task developed for the 8-arm radial maze. All training procedures were identical to those used in Experiment One.

Retention/Retraining. Following circadian re-entrainment procedures, subjects were tested for retention. Following retention testing, subjects were trained for nine additional days to reestablish asymptotic performance levels.

Extra-dimensional Set Shift. All procedures were identical to those used in Experiment One. Rats were trained for a period of 13 days, until asymptotic performance was achieved on this task.

Histology

Electron Microscopy

All procedures were identical to those used in Experiment One. The mean usable area for all samples was 99%, with scores ranging from 91 to 100%. Figures 4.2a and 4.2b are depictions of the areas selected from OPFC and HPC respectively.

Results

Circadian Rhythmicity

Phase-shifting and reentrainment. Daily running wheel activity was recorded over the course of the study. During the six days prior to phase-shifting rats began running shortly after light offset (overall number of minutes between light offset and activity onset, M = -23.41, SD = 50.25), with univariate ANOVA showing no main effects or interactions of sex and phase-shift. For a post-phase-shift measure for each of the four cycles, the average activity onset for the six days immediately following each phase-shift cycle was calculated. A univariate two way ANOVA (sex X phase shift) showed significant differences between phase-shifted (M= - 109.12, SD = 54.36) and non-phase-shifted rats (M = 3.66, SD = 33.19) for the second cycle, $F(1,13) = 21.80$, $p < .001$. A univariate two way ANOVA (sex X phase shift) showed significant differences between

phase-shifted (M= -107.78, SD = 58.05) and non-phase-shifted rats (M = -7.25, SD = 47.61) for the fourth cycle, $F(1,13) = 18.94$, $p = .001$. To ensure rhythm stabilization had occurred prior to retention testing, the average activity onset for the six days before retention testing was calculated. A univariate two way ANOVA (sex X phase shift) showed significant differences between phase-shifted (M= -196.57, SD = 32.68) and non-phase-shifted rats (M = -93.06, SD = 53.80), $F(1,13) = 27.76$, $p < .001$. Although there was a difference in the average onset, a repeated measures ANOVA, with degrees of freedom corrected using Greenhouse-Geisser due to sphericity violation, over the six days comprising this measure showed no interaction of day and phase shift, $F(3.011, 42.147) = 1.848$, $p = .153$. The lack of an interaction effect indicates that rhythm stabilization had occurred for both the phase-shifted and the non-phase-shifted groups, despite the differential activity onset.

Exercise Wheel Activity. Comparison of the activity profiles was conducted to determine whether running wheel activity patterns differed by sex of phase-shift. A repeated measures ANOVA revealed significant differences in activity by day $F(14,182) = 2.237$, $p = .008$. A significant interaction was observed between activity profile and sex because female rats (M = 8.94, SD = 1.03) were more active than male rats (M = 2.45, SD = 1.00), $F(14,182) = 2.384$, $p = .005$. In addition, a significant interaction was noted for activity profile and phase-shift because phase-shifted rats (M = 3.42, SD = .86) were less active than non-phase-shifted (M = 7.96, SD = 1.15), $F(14,182) = 1.951$, $p = .024$. A three-way interaction was observed across all the variables (i.e., activity profile, sex, and phase-shift) $F(14,182) = 1.847$ $p = .035$. These results indicate that observed differences

in behaviour may be attributed to the amount of exercise each animal is electing to perform.

Behavioural Assays

Morris Water Task

Acquisition

Trial duration. A five-way repeated measures ANOVA (sex X phase-shift X running wheel X day X trial) for trial duration revealed main effects of day and trial (*ps.* < .001) indicating that subjects learned over the course of MWT training. No significant main effects were observed for any of the other between-subject factors. A significant four-way interaction was observed for day, sex, phase-shift, and running wheel, $F(2.526, 83.347) = 3.988$, $p = .015$. All other interactions were not significant. Figure 3.1a represents the average of the total trial duration during acquisition of MWT per day broken down by phase-shift and sex. Figure 3.1b represents the average trial duration per day during acquisition for female rats only grouped by phase-shift and running wheel. Figure 3.1c represents the average trial duration per day during acquisition for male rats only grouped by phase-shift and running wheel. A univariate ANOVA of sex, phase-shift, and running wheel for total duration on the final training day revealed a significant interaction between sex and phase-shift, $F(1,33) = 10.216$, $p = .003$.

The analyses were also conducted excluding running wheel as a between subjects factor as was the case in Experiment One. A four-way repeated measures ANOVA (sex X phase-shift X day X trial) revealed significant main effects for day, $F(2.374, 52.326) = 112.661$, $p < .001$, trial $F(7, 154) = 8.193$, $p < .001$, and a significant two way interaction

between them, $F(7.762, 170.765) = 5.174$, $p < .001$. A significant three way interaction was observed for day, sex and phase-shift, $F(2.374, 115.005) = 3.850$, $p = .022$. A significant two way interaction was observed for trial and phase-shift $F(7, 154) = 3.044$, $p = .005$.

Path-length. A five-way ANOVA (sex X phase-shift X running wheel X day X trial) for path-length over the course of acquisition training revealed significant main effects of day and trial (*ps.* < .001). All other between-subject factors (phase-shift, sex, and running wheel) did not show a statistically significant difference. A significant four-way interaction was observed for day, sex, phase-shift, and running wheel, $F(2.114, 69.576) = 5.301$, $p = .006$. Figure 3.2a represents the average path length during acquisition of MWT per day grouped by phase-shift and sex. Figure 3.2b represents the average path length per day for female rats only during acquisition of the MWT grouped by phase-shift and running wheel. Figure 3.2c represents the average pathlength per day for male rats grouped by phase-shift and running wheel. A univariate ANOVA of sex, phase-shift, and running wheel for path-length on the final training day revealed a significant interaction between sex and phase-shift, $F(1,33) = 22.762$, $p < .001$.

The analyses was also conducted excluding running wheel as a between subjects factor as was the case in Experiment One. A four-way repeated measures ANOVA (sex X phase-shift X day X trial) revealed significant main effects for day, $F(2.132, 46.896) = 125.005$, $p < .001$, trial $F(7, 924) = 7.373$, $p < .001$, and a significant two way interaction between day and trial $F(7.385, 162.480) = 5.546$, $p < .001$. A significant three way interaction was observed for day, sex and phase-shift, $F(2.132, 46.896) = 6.043$, $p = .004$.

A significant two way interaction was observed for trial and phase-shift, $F(7,924) = 3.242$, $p = .003$.

Probe

Data for the probe trial was grouped into four separate bins labeled: bin one (full trial), bin two (0-10 seconds), bin three (10-20 seconds), and bin four (20-30 seconds).

Bin one (full trial). A repeated measures ANOVA assessing retention of the target quadrant, as indicated by the percentages of time spent in the target versus the average time spent in the other quadrants, revealed a interaction of quadrant, phase-shift, sex, and wheel, $F(1, 33) = 7.676$, $p = .009$. Figure 3.3a represents the dwell time in each quadrant for each group and shows the preference exhibited by each group for the target and other quadrants. One factor was eliminated from the interaction by taking the difference score between the target and other quadrant. Using the difference score, a univariate ANOVA revealed a three way interaction between phase-shift, wheel, and sex, $F(1,33) = 4.352$, $p = .045$. Subsequent pairwise comparisons revealed that the only group that did not differ for target preference was the phase-shifted males without wheels ($p = .001$). One-sample *t*-tests comparing the difference value to zero that revealed that only phase-shifted males without wheels failed to show a preference $t(2) = 1.001$, $p = .422$. The same analysis was conducted without running wheel and revealed a significant main effect of quadrant, $F(1,22) = 64.365$, $p < .001$. Post hoc, a repeated measures ANOVA comparing target (M = .41, SD = .10) to other quadrant (M = .20, SD = .03) revealed that all groups spent significantly more time in the target quadrant, $F(1,25) = 67.673$, $p < .001$.

Bin two (0-10). A repeated measures ANOVA assessing retention of the platform location, as indicated by the percentages of time spent in the target versus the average

time spent in the other quadrants, revealed no significant main effects or interactions, although an interaction between all variables approached significance, $F(1,33) = 4.099$, $p = .051$. Figure 3.3b represents the dwell time in each quadrant for the phase-shifted and non-phase-shifted rats for bin two. The same analysis was conducted without running wheel and revealed a significant interaction between quadrant and phase-shift, $F(1,22) = 4.297$, $p = .050$. Post hoc, a univariate ANOVA for target quadrant revealed that non-phase-shifted rats (M = .52, SD = .23) spent significantly more time in the target quadrant than phase-shifted rats (M = .34, SD = .17), $F(1,24) = 4.552$, $p = .043$.

Bin three (10-20). A repeated measures ANOVA assessing retention of the target quadrant, as indicated by the percentages of time spent in the target versus the average time spent in the other quadrants, revealed no significant main effects or interactions. The same analysis was conducted without running wheel and revealed a main effect for quadrant, $F(1,22) = 11.316$, $p = .003$. Post hoc, a univariate ANOVA revealed that all groups spent more time in the target quadrant (M = .42, SD = .24) than the other quadrants (M = .20, SD = .08), $F(1,25) = 11.693$, $p = .002$.

Bin four (20-30). A repeated measures ANOVA assessing retention of the target quadrant, as indicated by the percentages of time spent in the target versus the average time spent in the other quadrants revealed a significant interaction between quadrant and phase-shift $F(1,33) = 6.919$, $p = .013$. Post hoc, independent samples *t*-tests revealed that phase-shifted rats (M = .42, SD = .19) spent significantly more time in the target quadrant than non-phase-shifted rats (M = .26, SD = .17), $t(39) = 2.953$, $p = .005$. In addition, phase-shifted rats (M = .20, SD = .06) spent significantly less time in the other quadrants than the non-phase-shifted rats (M = .25, SD = .05), $t(39) = -2.789$, $p = .008$. Figure 3.3c

represents the dwell time in each quadrant for the phase-shifted and non-phase-shifted rats during bin four and shows the differential preference for the target quadrant exhibited by the phase-shifted rats during this final bin. The same analysis was conducted without running wheel and revealed a significant interaction between quadrant and phase-shift, $F(1,22) = 4.660$, $p = .042$. Post hoc, a univariate ANOVA revealed that the source of the interaction was that phase-shifted rats (M = .43, SD = .23) spent more time in the target quadrant than non-phase-shifted rats (M = .25 SD = .18), $F(1,24) = 4.986$, $p = .037$.

Visual Discrimination Task Using 8-arm Radial Maze

Acquisition

Days were grouped into a total of 14 blocks for analyses (Block one was training days one, two, and three; all other blocks were comprised of two subsequent days).

Choice Accuracy, Errors, and Re-entry Performance. In order to ensure that all groups acquired the task in a similar manner, three four-way ANOVAs were performed (sex X phase-shift X running wheel X block) for choice accuracy, percentage of errors, and percentage of reentries. A main effect was observed for block ($p < .01$). No other significant main effects or interactions were observed. Figures 3.4a, 3.5a, and 3.6a represent the choice accuracy, percentage of errors, and percentage of re-entries exhibited by rats grouped by phase-shift and sex during acquisition of the visual discrimination task using the 8-arm radial maze. Figures 3.4*b* and *c*, 3.5*b* and *c*, and 3.6*b* and *c* represent the performance of females and males respectively for overall performance, proportion of errors, and proportion of re-entries grouped by phase-shift and wheel. All figures show the similar fashion with which all groups acquired the task. The same analyses were run

without wheel and did not reveal any main effects or interactions for the between-subject factors.

Retention: Comparison of final training day to first retention day

Choice Accuracy. To test retention, a repeated measures ANOVA was performed comparing choice accuracy and the final training day to the first retention day. A significant three-way interaction for day, sex, and phase-shift, $F(1,33) = 6.117$, $p = .019$, was observed. Figures 3.7a represents the comparison of the average performances on the last training day and the first retention day including sex and phase-shift as factors. Figure 3.7b represents the performance of the female rats grouped by phase-shift and wheel condition comparing the final training day and the first day of retention testing. Figure 3.7c represents the performance of male rats grouped by phase-shift and wheel condition comparing the final training day and the first day of retention testing. These figures reveal the large decrease in performance accuracy exhibited by the phase-shifted males, particularly those without wheels. Figure 3.7d shows the degree to which the phase-shifted males, especially those without wheels, differ from the other groups. A univariate ANOVA of overall performance revealed that for phase-shifted rats, females (M = .83, SD = .20) had significantly better retention than did males (M = .37, SD = .41), $F(1,17) = 10.076$, $p = .006$. A repeated measures ANOVA excluding wheel as a between-subjects factor revealed a significant interaction between day and sex, $F(1, 22) = 6.062$, $p = .022$. Post hoc, a univariate ANOVA for the calculated score of the first retention day minus the final training day revealed that females (M = -.05, SD = .21) performed better than males (M = -.37, SD = .42), $F(1,24) = 5.909$, $p = .023$.

Error. To test retention, a repeated measures ANOVA comparing the proportion of errors on the last training day and the first retention day was performed. A significant interaction was observed among day, sex, phase-shift and wheel, $F(1,33) = 4.766$, $p = .036$. Figure 3.8a represents the comparison of the percentage of errors made by each group on the final training day and the first day of retention testing grouped by sex and phase-shift. Figure 3.8b represents the comparison of the percentage of errors made by female rats only on the final training day and the first retention day grouped by phase-shift and wheel. Figure 3.8c represents the comparison of the final training day to the first retention day for male rats only grouped by phase-shift and wheel. This figure shows the differential performance of the phase-shifted males without wheels. Figure 3.8d represents the percentage of errors for each group on the first day of retention testing and reveals the substantially higher percentage of errors made by the phase-shifted males without wheels. A post hoc univariate ANOVA for the percentage of errors on the first day of retention testing revealed a significant interaction between sex and wheel, $F(1,33) = 4.680$, $p = .038$. A univariate ANOVA revealed a significant difference between male rats with running wheels (M = .14, SD = .13) and those without (M = .31 SD = .15), $F(1,18) = 6.653$, $p = .019$. Further univariate ANOVA revealed that for rats with running wheels, females (M = .13, SD = .14) performed significantly better than males (M = .31, SD = .15), $F(1,13) = 5.620$, $p = .034$. A repeated measures ANOVA excluding running wheel as a between subjects factor revealed a main effect of day ($p = .031$) because rats made more errors on the first day of retention testing (M = .09, SD = .13) than the final day of training (M = .17, SD = 17), $F(1,25) = 5.319$, $p = .030$. No significant interactions were observed.

Re-entry. To test retention, a repeated measures ANOVA comparing the percentage of re-entries on the last training day and the first retention day was performed. A significant interaction between day and sex, $F(1,33) = 8.573$, $p = .006$ was observed. All other interactions were non-significant. Figure 3.9a represents the comparison of the percentage of re-entries made during the final training day and the first retention day grouped by sex and phase-shift. Figures 3.9b represents the comparison for female rats only on the final training day and the first retention day grouped by phase-shift and running wheel. Figure 3.9c represents the same comparison for re-entries on the final training day to the first day of retention testing for male rats grouped by phase-shift and running wheel. Figure 3.9d represents the performance of each group on the first day of retention testing and shows the larger proportion of perseverative errors made by the phase-shifted male rats. A post hoc univariate ANOVA revealed a significant main effect of sex because females (M = .03, SD = .06) made fewer reentries than males (M = .28, SD = .28), $F(1,33) = 15.185$, $p < .001$. No other significant main effects or interactions were noted for the number of re-entries on the first day of retention testing. A repeated measures ANOVA excluding running wheel revealed a significant interaction between day and sex, $F(1,22) = 11.254$, $p = .003$. Post hoc, a univariate ANOVA for the calculated score of the first retention day minus the final training day revealed that females (M = -.03, SD = .09) performed better than males (M = .27, SD = .31), $F(1,24) = 11.260$, $p = .003$.

Reestablishment of asymptotic performance

Days were grouped into a total of five blocks for analyses (all blocks were comprised of two subsequent days).

Choice Accuracy. Total performance across retention days was analyzed using repeated measures ANOVA. A significant three way interaction was observed for block, sex, and phase-shift, $F(4,132) = 3.640$, $p = .008$. Significant between subject effects were observed for sex, $F(1,33) = 5.370$, $p = .027$ and wheel, $F(1,33) = 7.036$, $p = .012$. Post hoc analyses revealed that across testing, female phase-shifted rats were significantly different from female non-phase-shifted rats, $F(4,76) = 4.246$, $p = .004$. Independent samples *t*-tests revealed for female rats only, phase-shifted rats (M = .92, SD = .10) outperformed non-phase-shifted rats (M = .77, SD = .18) on retention block four, $t(16.129) = 2.360$, $p = .031$. All other main effects and interactions were not significant. Figure 3.10a represents the reestablishment of asymptotic performance following phase-shifting on choice accuracy for rats grouped by sex and phase-shift. Figure 3.10b represents the performance of female rats across the five training blocks grouped by phase-shift and running wheel. Figure 3.10c represents the performance of male rats across the five retraining blocks grouped by phase-shift and running wheel, showing the marked differences in performance, particularly by phase-shift. A repeated measures ANOVA excluding wheel revealed a significant effect of day, $F(4, 88) = 8.277$, $p < .001$.

Error. Error performance across retention test days was analyzed using repeated measures ANOVA. A significant three way interaction for block, sex, and phase-shift, $F(4,132) = 2.801$, $p = .029$, was observed. For the third trial block, a post hoc univariate ANOVA revealed that females (M = .11, SD = .10) made fewer errors than males (M = .18, SD = .14), $F(1,37) = 4.873$, $p = .034$. A post hoc univariate ANOVA on the fourth trial block revealed a significant interaction between sex and phase-shift, $F(1,37) = 5.935$, $p = .020$. Post hoc univariate ANOVAs revealed that phase-shifted males (M = .18, SD =

.15) made more errors than both non-phase-shifted males (M = .06, SD = .08), $F(1,18) = 4.954$, $p = .039$, and phase-shifted females (M = .06, SD = .08), $F(1,18) = 5.506$, $p = .031$. Figure 3.11a represents the percentage of errors made by rats during the reestablishment of asymptotic performance level grouped by sex and phase-shift. Figure 3.11b represents the percentage of errors across the five training blocks for female rats grouped by phase-shift and wheel. Figure 3.11c representing the percentage of errors across the five training blocks for male rats grouped by phase-shift and wheel shows the poor performance of the phase-shifted males without wheels relative to the other groups. A repeated measure ANOVA excluding wheel revealed a significant effect of day, $F(4, 88) = 8.976$, $p < .001$.

Re-entry. Re-entry performance across retention test days was analyzed using repeated measures ANOVA. A significant interaction was observed for block and sex, $F(2.045, 67.482) = 5.660$, $p = .005$. Independent samples *t*-tests revealed that for retention block one, females (M = .07, SD = .07) made significantly fewer re-entries than males (M = .29, SD = .31), $t(20.927) = 3.121$, $p = .003$. All other interactions were not significant. Figure 3.12a represents the percentage of re-entries during the reestablishment of asymptotic performance levels for rats grouped by sex and phase-shift. Figure 3.12b represents the percentage of re-entries across the five training blocks for female rats grouped by phase-shift and running wheel. Figure 3.12c represents the percentage of re-entries across the five training blocks for male rats grouped by phase-shift and running wheel. It is clear that the only block to show marked differences in performance levels was the first block. This reveals that rats were able to reestablish asymptotic performance levels. A repeated measures ANOVA excluding wheel revealed

a significant interaction between day and sex, $F(4, 88) = 8.857$, $p < .001$. This effect emerges because males made significantly more reentries during the first, $F(1, 24) = 8.720$, $p = .007$, and second blocks $F(1, 24) = 5.709$, $p = .025$.

Extra-dimensional Set Shift

Days were grouped into six blocks for analyses (blocks one through five were comprised of two subsequent days whereas block six was comprised of the final three days). To ensure that the rats were responding to the switch in cue, the first selection on the final training day was compared to the first selection on the final EDS training day using a repeated measures ANOVA. No significant main effect of cue type was observed ($p = .070$), nor were there any interactions with sex ($p = .610$), phase-shift ($p = .645$), or running wheel ($p = .950$).

Choice Accuracy. A repeated measures ANOVA for performance across the extra-dimensional shift condition revealed no significant main effects or interactions of day, sex, phase-shift, or running wheel. Figure 3.13a represents the choice accuracy across extra-dimensional set shift training for rats grouped by sex and phase-shift. Figure 3.13b represents the choice accuracy across the six training blocks for female rats grouped by phase-shift and running wheel. Figure 3.13c represents the choice accuracy across the six training blocks for male rats grouped by sex and running wheel. A repeated measures ANOVA excluding wheel revealed a significant three way interaction between day, sex, and phase-shift, $F(5, 110) = 2.679$, $p = .025$. Post hoc, a repeated measures ANOVA revealed differences between phase-shifted males and females across EDS training, $F(5, 50) = 2.674$, $p = .034$.

Error. A repeated measures ANOVA for the percentage of errors during the extra-dimensional shift condition revealed a significant interaction of block, phase-shift, and wheel, $F(5,165) = 2.389$, $p = .040$. For phase-shifted rats, there was a significant interaction between block and wheel, $F(1,17) = 5.148$, $p = .037$. A post hoc univariate phase-shift by wheel ANOVA revealed that rats with running wheels (M=.25, SD = .17) made significantly more errors than those without (M = .16, SD = .09) during block two, $F(1,37) = 3.147$, $p = .027$. A post hoc univariate phase-shift by wheel ANOVA revealed that phase-shifted rats (M=.14, SD = .11) made significantly more errors than non-phase-shifted rats (M = .07, SD = .08) during block five, $F(1,37) = 5.477$, $p = .025$. A post hoc univariate phase-shift by wheel ANOVA revealed that phase-shifted rats (M=.10, SD = .08) made significantly more errors than non-phase-shifted (M = .06, SD = .06) during block six, $F(1,37) = 5.791$, $p = .021$. A three-way between subjects factor interaction was observed for sex, phase-shift, and wheel $F(1,33) = 4.173$, $p = .049$. Post hoc, a univariate ANOVA revealed a significant interaction between phase-shift and wheel for females, $F(1,17) = 5.894$, $p = .027$. An additional post hoc univariate ANOVA revealed that for rats with running wheels, phase-shifted rats (M = .22, SD = .15) made significantly more errors than non-phase-shifted rats (M = .15, SD = .07), $F(1,17) = 5.235$, $p = .032$. These results indicate that phase-shifted rats with running wheels were impaired at switching their behaviour based on changes in the reinforcement contingencies. Figure 3.14a, representing the percentage of erroneous entries across EDS training for rats grouped by sex and phase-shift, shows that phase-shifted rats made more errors during the fifth block. Figure 3.14b, representing the percentage of errors made across the six training blocks for female rats grouped by phase-shift and running wheel, shows the relatively

poor performance of the female phase-shifted rats on the second block. Figure 3.14c represents the percentage of errors made across the six training blocks for male rats grouped by phase-shift and wheel. A repeated measures ANOVA excluding running wheel as a factor revealed a main effect of EDS training day, $F(5, 110) = 11.429$, $p < .001$.

Re-entry. A comparison of groups for the proportion of re-entries during the extra-dimensional shift condition revealed no significant main effects or interactions. Figure 3.15a, representing the percentage of re-entries made across the six EDS training blocks for rats grouped by phase-shift and sex, shows the similar performance of all groups. Figure 3.15b represents the percentage of re-entries made across the six training blocks for female rats grouped by phase-shift and running wheel. Figure 3.15c represents the percentage of re-entries made across the six training blocks for male rats grouped by phase-shift and running wheel. A repeated measures ANOVA excluding running wheel as a factor revealed a main effect of EDS training day, $F(5, 110) = 5.090$, $p < .001$.

Histology

Electron Microscopy

Dorsal Hippocampus. A univariate ANOVA (sex X phase-shift) for the number of mitochondria in the HPC revealed no significant main effects or interactions. Figure 3.16a represents the average density of mitochondria in HPC (see figure 4.4 for an example photomicrograph).

Prefrontal Cortex. A univariate ANOVA (sex X phase-shift) for the number of mitochondria in OPFC revealed no significant main effects or interactions. Figure 3.16b

represents the average density of mitochondria in OPFC (see figure 4.5 for an example photomicrograph).

Discussion

Assessment of previously acquired hippocampal and dorsal striatal associations was conducted using the MWT and 8-arm radial maze, respectively. Rats were trained to asymptote and then phase-shifted. Following circadian rhythm stabilization retention of the platform location on MWT and the S-R association on 8-arm radial maze were tested. The ability to perform an EDS on the 8-arm radial maze was assessed after the completion of retention testing.

The strength of the preference for the target quadrant on the MWT differed by group. Non-phase-shifted males without wheels showed the strongest preference whereas phase-shifted males without wheels showed the lowest degree of preference for the target quadrant. In fact, phase-shifted males without running wheels did not show a preference for the target quadrant. For performance on the visual discrimination task, groups differed on how well they retained the memory supporting this learned behaviour. Interestingly, females exhibited superior retention on all three measures (choice accuracy, percentage of errors, and percentage of re-entries) of performance on this task. Although females exhibited better retention than both groups of males as measured by percentage of errors, males with running wheels retained the memory supporting this behaviour better than those without.

Over the course of retraining on the 8-arm radial maze, a significant interaction of block, sex, and phase-shift was observed. When all days were assessed simultaneously, the source of the interaction appeared to be that phase-shifted females outperformed non-phase-shifted females. For choice accuracy by block, phase-shifted females out-performed non-phase-shifted females on training block four. For the percentage of errors, during the third trial block, females outperformed males. Also, on the fourth trial block, non-phase-shifted males outperformed phase-shifted males while phase-shifted females continued to outperform phase-shifted males. For the percentage of reentries during the first trial block, females outperformed males. During EDS training, rats with running wheels did better than those without, on the second training block and phase-shifted rats did better than non-phase-shifted rats on the fifth and sixth training blocks. In general, non-phase-shifted rats outperformed phase-shifted rats and females did better than males at re-establishing asymptotic performance levels. Although the effects across block are relatively muddled, it appears as though chronic phase-shifting produces negative impacts on cognitive performance involving dorsal striatum. No significant differences were observed for mitochondrial density using electron microscopy.

Morris Water Task. With the exception of the phase-shifted males without wheels, all groups showed a preference for the target quadrant over the total duration of the probe test. Groups also differed during the final 10 seconds of the probe trial. The source of this difference was the phase-shifted rats spending more time in the target quadrant, revealing a possible impairment in behavioural flexibility. The finding that males, but not females, are affected by chronic phase shifting is worthy of further interpretation and investigation. Females must endure a relatively high degree of

variation in circulating hormone levels across circadian and estrus cycles (Mahoney et al., 2004; Perrin et al., 2006) and inconsistent circadian rhythms exhibited during pregnancy (Okun & Coussons-Read, 2007). Therefore, there is pressure to develop strategies or mechanisms for compensating that could be providing some degree of protection from the effects of the phase shift. The effect of running wheel for males implies that exercise may have protective effects against phase-shifting. One possible explanation for the difference is that male and female rats are using running wheels differently. Analyses did reveal significant differences in running wheel activity by sex indicating that exercise may be a contributing factor. However, the persistence of differences on the behavioural tasks during the acute phase-shift condition (i.e., when no difference in activity profile was observed), indicate that exercise amount is only a contributing factor.

Visual Discrimination Radial Maze. These findings suggest that phase-shifting produces differential effects on retention by sex, particularly in the absence of a running wheel. Previous reports regarding sex differences in retention are inconsistent, although it is typically reported that males have an advantage (for review, see Jonasson, 2005). However, these findings indicate superior retention in females for choice accuracy, percentage or errors, and percentage of reentries. Exercise and sex appear to impact retention more than phase-shifting, although this observation was not in the predicted direction for sex.

The finding that behavioural flexibility was impaired during performance of the extra-dimensional set shift, suggest that PFC is affected by chronic circadian disruption. The decreased ability to perform the extra-dimensional set shift exhibited by the phase-shifted animals indicates that circadian disruption either (1) negatively impacts

behavioural flexibility, or (2) strengthens the original association to such a degree that it becomes difficult for the rats to alter their strategy to accommodate changes in reinforcement contingency effectively. However, that the phase-shifted rats did not show superior retention during re-testing makes the latter less tenable.

Electron microscopy. At the time point used in this experiment, mitochondrial density in HPC and OPFC was unaffected by chronic phase-shifting. It is entirely possible that the number of mitochondria in these regions is only transiently affected by circadian disruption and had observation occurred prior to re-entrainment or at various time points within the phase-shifting cycle, changes could have been observed. An alternative hypothesis is that circadian disruption affects other regions and mitochondrial density in HPC and PFC is largely unaffected by circadian disruption.

Implications

Results indicate that chronic phase-shifting impairs retention on MWT and 8-arm radial maze. In addition, chronic phase-shifting impairs the ability to perform an EDS, although this effect is less robust than the retention effects. Chronic phase-shifting appears to elicit differential effects in male and female rats. Male rats appear to be more deeply affected by chronic phase-shifting, especially when performing the MWT. This finding is alarming given the increase in males entering professions where shift-work is the norm such as nursing (Evans, 2004). In addition, running wheel activity appears to mediate the effects of phase-shifting, especially in males. Possibly the most important implication of the present results is that impairments persist beyond circadian rhythm stabilization. The permanence of these impacts is alarming and should encourage policy makers to reevaluate their scheduling practices as recommended in Chapter Two.

Future Directions and Limitations

Many of the recommendations put forward in Chapter Two still apply. It could be the case that changes to the brain may be gross, such as changes in volume, connectivity, or receptor density. The impairments associated with the extra-dimensional set shift are slight and it would be interesting to examine the impacts of altering the reinforcement contingency in a more drastic fashion (e.g., complete reversal or the use of novel cues). The difference in the amounts of running wheel activity should be controlled in future experiments to eliminate the possibility that exercise amount is the source of the sex difference. Unfortunately, doing so is also confounded because the amount of activity performed by male and female rats differs in healthy animals. In other words, restricting females from accessing running wheels may produce independent effects. The finding that exercise wheel ameliorates the effects of phase-shifting implicates cellular processes including neurogenesis, apoptosis, and cell survival. Therefore, future research should focus on the rates of the cellular markers related to these processes.

Conclusion

Chronic circadian disruption can negatively impact memory retention on spatial and visual discrimination tasks as well as behavioural flexibility on both MWT and the visual discrimination task on the 8-arm radial maze. It appears that while phase-shifting produces impairments, there are interesting sex differences. Electron microscopy analysis of mitochondrial density failed to reveal evidence of differences between groups although behavioural impairments were observed. However, the emergence of the behavioural impairments suggests some physical basis for these impairments, although differences in mitochondrial morphology, hence metabolism, are likely not the basis.

CHAPTER FOUR: GENERAL DISCUSSION

As detailed in Chapter One, phase-shifting alters the function of the SCN. Naturally occurring alterations in the function of the SCN are associated with disease states, including mood disorders (Benca et al., 2009; Mendlewicz, 2009), various dementias (Antoniadis et al., 2000; Bombois et al., 2010), and cancer (Blask et al., 2005; Pyter et al., 2010). Each of these disease states is accompanied by a particular constellation of cognitive impairments, which often relate to learning, memory, and attention (Craig & McDonald, 2008; Pyter et al., 2010). In the present experiments, the light/dark schedule was repeatedly adjusted thereby disrupting the SCN. The phase-shift induced arrhythmicity in the SCN of otherwise healthy young rats provided a model for studying the impacts of circadian disruption independent of other factors associated with various disease states (see above). In addition, by employing phase-shifting schedules, one can examine the effects of circadian disruption independently, as a model of jet lag or shift work, or a component of a disease state (Reddy et al., 2002; Selgado-Delgado et al., 2008).

Based on the available evidence, as reviewed in Chapter One, it was reasonable to hypothesize that phase-shifting would produce cognitive impairments, particularly in functional domains dependent on HPC and PFC. In the experiments detailed in Chapters Two and Three, this hypothesis was tested by examining the impacts of acute and chronic phase-shifting on the behaviour of male and female rats. Several tasks that are critically dependent on distinct neural regions were employed following circadian rhythm re-entrainment. Specifically, the Morris Water Task that was used to assess hippocampal

function and a visual discrimination task using the 8-arm radial maze was used to assess both dorsal striatal and PFC function.

Distinguishing between Orbital and Infralimbic/Prelimbic PFC during EDS Performance

Traditionally, tasks assessing EDS ability measure dimensions such as impulsivity (e.g., go-no go paradigms; Winstanley, 2007) during Response-Outcome (R-O) associations, the execution of which is likely to arise from I/P PFC given its excitatory input to downstream structures (Ostlund & Balleine 2007). Conversely, this EDS task requires the attentional shift of an S-R association. Thus, it was presumed that this EDS task is dependent on an additional/different PFC subregion, the OPFC. The OPFC exhibits bilateral connectivity with, and may inhibit activation of, structures related to arousal and response patterns to previously acquired complex associations (e.g., amygdala, hippocampus, dorsal striatum). Proper execution of this EDS task requires inhibition of aspects of the previously acquired association. Previous reports that OPFC is dampening responding to extinguished associations (Zelinski et al., 2010) lends support to the notion that OPFC, not I/P PFC, is regulating the alteration in response pattern during performance of this task.

A previously acquired S-R association is well developed, and the distinction between an R-O and S-R association is that overtraining is thought to have occurred during the latter (Ostlund & Balleine 2007). Modification of S-R associations is complex due to their multidimensional nature. The ability to adjust an association in an identical environment is two-dimensional. First, detection and appropriate response to the change in reinforcement contingencies (i.e., increased excitation of the secondary association) is

necessary. This neural component of the EDS is likely to reside in I/P PFC. However, the controlled inhibition of the primary association (i.e., the original association) is equally important. It is this inhibitory role that is thought to be important for the execution of this task.

The rationale behind the assumption is that it is the active inhibition that is necessary for the performance of this EDS, not the increase in the specificity of excitation, is that during performance of the task the rat can continue to respond more strongly to the original, or primary, association as re-entry is not blocked. In other words, the rat could continue to enter only the lit arms, effectively ignoring the panel cue for the duration of training. It is the inhibition of the tendency that is dependent on OPFC. Thus, OPFC is contributing to the proper execution of this particular EDS task.

Summary of Results

Morris Water Task (MWT)

A variant of the MWT was used to assess hippocampal function. In order to successfully perform this task the rat must use cues found in the testing space to determine the location of a platform hidden under the surface of the water. Training ceased when all groups were able to locate the target platform quickly and without difficulty, indicating that prior to phase-shifting, groups were performing in a similar fashion. Following phase-shifting and circadian re-entrainment, memory for the platform location was tested. Successful retention, measured by comparing the time spent in the target quadrant to the other quadrants, is indicative of proper hippocampal function.

Retention testing on MWT, following phase-shifting and circadian reentrainment revealed that, over the entire duration of the probe trial, acute phase-shifting impaired retention. The acutely phase-shifted rats spent significantly less time in the target quadrant than the non-phase-shifted rats. In particular, during the first ten seconds of the probe trial for acutely phase-shifted rats and controls, phase-shifted rats spent significantly less time in the target quadrant than the non-phase-shifted rats. In addition, female rats showed a greater preference for the target quadrant than males.

Behavioural patterns on MWT for the chronic phase-shift experiment differed from those observed in the acute phase-shifting experiment. All groups showed preference for the target quadrant over the entire trial duration when analyses included only phase-shift and sex as between subject factors. The effect was strengthened by the inclusion of running wheel as a between-subjects factor. Including wheel as a factor, phase-shifted males without wheels show a markedly different behavioural profile than the other groups, they were severely impaired. In addition, chronically phase-shifted groups spent significantly more time in the target quadrant than non-phase-shifted groups during the final 10 seconds of the probe trial. This type of perseverative error could indicate a decline in behavioural flexibility.

Visual Discrimination Task Using the 8-arm Radial Maze

A visual discrimination task using the 8-arm radial maze was employed to assess functioning of the dorsal striatum and PFC. Prior to phase-shifting, four of the eight arms were delineated from the others by a lit LED light strip and reinforced with a food reward. Two of the four lit arms also contained a textured floor panel (see figure 4.1a). Rats were trained on the task until they achieved an 80% success rate (i.e., asymptote; an

average of four correct entries in five) for three consecutive days. Following phase-shifting and re-entrainment rats were tested for retention of the original reinforcement schedule (i.e., four lights with two panels). Following this test day, rats were retrained until asymptotic performance was re-established. This first phase of radial maze performance assessed function of the dorsal striatum (Featherstone & McDonald, 2004; Featherstone & McDonald, 2005; McDonald & Hong, 2004).

After asymptotic performance levels were reestablished, the reinforcement contingencies of the cues were reversed. That is, four of the eight arms were delineated from the others by the presence of textured floor panels that served as the cue for the food reward. Two of the four arms containing textured panels also contained a lit LED light strip (see figure 4.1b). The purpose behind reversing the cues was to elicit an EDS. This second phase of radial maze testing was dependent upon function of the PFC during which it must identify the switch in reinforcement contingency and respond accordingly.

Acute circadian disruption alone did not produce any effects. However, when retention was analyzed in the acute phase-shift condition, males exhibited better retention than females. Observations on the pharmacological differences by sex have been noted in striatum, but the apparent male advantage on the 8-arm radial maze is among the first observations of sex differences during behaviour dependent on the dorsal striatum. All groups of rats in the acute phase-shift condition were equivalent with respect to their performance on the extra-dimensional set shift.

During the chronic phase-shift experiment, a number of patterns were observed. First, phase-shifting appeared to negatively impact the performance of males, but not females. This effect was exaggerated among males without running wheels, whereby

non-phase-shifted rats exhibited better retention than phase-shifted rats. In addition, phase-shifted males made more errors than both the non-phase-shifted males and the phase-shifted females during performance of the 8-arm radial maze. Females made fewer reentries than males for the first block of retraining, and made fewer errors during the third training block. These findings suggest that phase-shifting did not impact males and females in a similar fashion. This would not be the first observation of differential effects by sex. In fact, differences in the expression of dendritic spines of PFC in male and female rats in response to stress have been noted (Shors et al., 2001).

Histology

Analysis of mitochondrial morphology by electron microscopy did not produce any significant effects of phase-shifting.

Interpretation

Morris Water Task

The Morris Water Task, and its many variants, is commonly held as the gold standard by which all other spatial and navigational tasks are measured (D'Hooge & De Deyn, 2001). When performing the MWT, rats use proximal and distal cues to navigate to a hidden platform location. The ability to navigate to the platform means the rat can incorporate cues from a variety of dimensions into a cohesive representation that can be used to successfully navigate to the hidden target.

In order for analyses to be performed, the MWT was divided into four equal quadrants. One of the four quadrants contained the target platform, which was the only escape point from the pool. A preference for this quadrant (i.e., the target quadrant) is

thought to represent memory for the platform location (for review, see Sutherland et al., 1982). Degree of preference for a particular quadrant is indicated by dwell time in that quadrant versus the other quadrants. However, when the platform is removed, a normal rat will learn that the target platform has been moved and widen the search area to include other quadrants. When the rat fails to adapt in this fashion, it is categorized as a perseverative error. Errors in perseveration are thought to occur due to a lack of behavioural flexibility.

Retention testing on MWT revealed that, over the entire duration of the probe trial, acute phase-shifting impaired retention in both sexes, as indicated by a stronger preference for the target quadrant in non phase-shifted versus phase-shifted rats. Conversely, for the chronic phase-shift experiment, all groups showed preference for the target quadrant over the entire trial duration, with the exception of the phase-shifted males without wheels. In addition, chronically phase-shifted rats limited the majority of their search to the target quadrant during the final 10 seconds of the probe trial. These three findings bear on the hypothesis assessed here and, therefore, warrant further discussion.

All phase-shifted groups in the acute phase-shift experiment showed behavioural impairment whereas this was not the case in the chronic phase-shifting experiment. One possible explanation for these differences between the experimental outcomes is that under chronic phase-shift conditions the rats adjusted to the circadian disruption and were thus better able to cope. Unfortunately, this cannot be definitively concluded because the intervals between training and testing were different across experiments. Future research could address this shortcoming by using equivalent inter-training phase intervals.

The finding that the only group to exhibit retention impairments in the chronic phase-shifting experiment was the phase-shifted males without running wheels can be interpreted in a number of ways. It could be the case that access to running wheels is somehow protective against the effects of phase-shifting. The protective effects of exercise are well documented in other paradigms (e.g., stroke; Marin et al., 2003) and could be ameliorating the effects of phase-shift as well. No differences were noted for exercise amount during the acute condition and behavioural effects still emerged indicating that, although running wheel activity may be contributing, it is not responsible for the observed behavioural impairments exclusively.

Circadian rhythm disruption is associated with elevated levels of glucocorticoids (Dickmeis, 2009). In addition, strong relationships exist between glucocorticoids and androgens (Viau, 2002), and androgens and growth hormones (Yang et al., 2004). Furthermore, strong relationships exist between all of these factors and rates of neurogenesis (Galea et al., 2008; Galea, 2008; Melvin et al., 2007). Although the formation of new cells may be important for normal function, the survival of those cells is equally, if not more, important. Exercise might be potentiating the rate of survival in new neurons thereby alleviating behavioural impairments (Lafenetre et al., 2009). The observation of impairments in males without running wheels could be an emergent property of these relative phenomena, although there are several possible routes through which these behavioural impairments could have emerged.

One possibility is that phase-shifting is leading to a loss of cells. Stereological investigation of cell density or cells expressing proteins related to apoptotic processes (e.g., caspase-3; Broughton et al., 2009) could quickly identify whether this is occurring,

especially if conducted at various time points across the phase-shifting cycles. The greater degree of impairment during MWT performance exhibited under acute phase-shifting relative to the chronic phase-shift condition indicates that there may be a parabolic relationship between phase-shift duration and rates of neurogenesis, apoptosis, and cell survival. Another possibility is that the behavioural effects are driven by alterations in signaling pathways such as nerve growth factor (NGF; Sofroniew et al., 2001).

A related alternative is that phase-shifting is leading to a decrease in neurogenesis and that continued renewal of cells within HPC are critical for normal memory function, as is the case with sleep fragmentation (Guzman-Marin et al., 2008). In fact, impacts of circadian disruption on adult neurogenesis have already been reported (Meerlo et al., 2009). The finding that the effect of circadian dysfunction is both attenuated in females and enhanced in rats without running wheels lends support to the role of neurogenesis in the observed impairments.

The hypothesis that the effect is being driven by neurogenesis can also explain the observed sex differences. The expression of Neu-N, a marker of neurogenesis, shows a marked sex difference (Saucier et al., in prep). Over time, females exhibit a greater increase in the density of cells within the DG than do males. It could be that the differential neurogenesis in males and females could be driving the effect. That is, the higher rates of neurogenesis observed in females relative to males leads to a decrease in the necessity of potentiating the survival of new neurons given that females have an overabundance of new cells relative to males. In other words, phase-shifted female rats do not appear to need running wheel access to protect them from the functional impacts

of phase-shifting. Alternatively, if rates of neurogenesis are equal across sex, it could be the case that the elevated exercise exhibited by females in the chronic phase shift condition is increasing the survival rates of newly formed neurons.

The third finding to be interpreted is the perseveration exhibited by chronically phase-shifted rats during the final bin of the probe trial. Groups did not differ during the middle third of the trial. As highlighted above, perseveration is indicative of impairments in behavioural flexibility. That is, it appears that the non-phase-shifted rats widened their search for the platform to other quadrants whereas the phase-shifted rats failed to do so. Given these findings, further examination of impairments in behavioural flexibility on hippocampal and non-hippocampal dependent tasks is warranted (Zelinski et al., 2010).

Sex differences in response to chronic phase-shifting

As stated previously, chronic phase-shifting produced marked differences in male and female rats. Male rats were more deeply affected by chronic phase-shifting. Although several hypotheses can be generated regarding the basis and implications, it may be that the evolutionary pressures the female circadian clock faced have led to the development of a more resilient system in females, and because there is reduced necessity in males, there is little selection pressure for resilient circadian clocks. There are several tasks that females complete that endanger the circadian clock, the most notable of which is childcare. Circadian rhythm disruption has been observed during pregnancy (Okun & Coussons-Read, 2007) and infants require constant care, a demand that is known to place strain on the sleeping habits of the mother, both rat and human (Dennis & Ross, 2005). Future investigations could investigate the role of androgens by examining the effects of

circadian disruption on rats gonadectomized at various points across the life cycle (e.g., infancy, postpubertally, or in adulthood).

In addition to the sex differences in response to phase-shifting, males and females responded differently to the absence of running wheels. The finding that not having a running wheel significantly increased impairments on DS and HPC dependent tasks in males, but not females, is a finding worthy of additional investigation. Studies could examine these phenomena by examining androgen-compromised subjects either lacking sex-typical hormones, or with increased hormones of the opposite sex. Furthermore, gonadectomizing rats at various life points (e.g., neonatally, prepubertally, postpubertally) could provide additional information about how sex hormones may be interacting with exercise and circadian disruption to produce the observed behavioural impairments.

Visual Discrimination Task Using the 8-arm Radial Maze

The modified version of the S-R radial maze employed here is a variant of the visual discrimination task employed by McDonald & White (1993). The 8-arm radial maze can be adapted such that different structures are critical given the paradigm employed (McDonald et al., 2008). McDonald and White have shown that the manipulation of the stimulus conditions leads to differential contributions of the striatum, HPC, PFC, and amygdala (McDonald & White, 1993). In addition to the contributions of striatum, the visual discrimination variant of the S-R radial maze employed here is dependent on the PFC. In order to complete this version of the task, the rat must respond properly to a switch in the reinforcement contingencies of the cues. In this case, it involved switching attention from a light to a textured panel. Successful performance of

this task indicates that the rat was able to acquire the original association, retain the association, and note the change in reinforcer saliency when the reinforcement conditions of the stimuli were reversed. In addition to learning these associations, the rat must also learn not to revisit arms it has previously entered. The inclusion of this EDS is how PFC function was examined using the 8-arm radial maze.

Retention. Sex differences in retention have been reported on the visual discrimination task on the radial maze and the present findings from the acute phase-shifting experiment replicate what has been previously reported, with males exhibiting better retention than females (for review, see Jonasson, 2005). However, the findings of the chronic experiment failed to support the sex difference. In fact, the opposite was true, with females exhibiting superior performance relative to males for error and reentry rates.

In addition, phase-shifting appeared to negatively impact the performance of males during retention testing. The effect was strongest among males with running wheels, revealing that non-phase-shifted rats exhibited better retention than phase-shifted rats. In addition, phase-shifted males made more errors than both the non-phase-shifted males and the phase-shifted females. In contrast, there was some evidence to suggest that phase-shifting did not similarly impair females. Female phase-shifted rats performed better than female non-phase-shifted rats on the fourth retraining block. Regardless, the overall pattern that emerged among rats with running wheels in the chronic experiment is that phase-shifting impairs retention.

Retraining. Under acute phase-shift conditions all groups reestablished asymptotic performance levels within three days, whereas it took 10 days for all groups in the chronically phase-shifted condition to re-establish an 80% success rate.

Unfortunately, direct statistical comparison of the two groups is confounded given the differences in the number of training days and the interval between training and testing. Given that all groups exhibited the same behavioural patterns within each experiment, it is likely the case that the difference in the interval between training and retraining is driving the effect. This possibility could be addressed in an experiment by equating the inter-training intervals.

Extra-dimensional set shift. Taken together, the results from 8-arm radial maze indicate that acute phase-shifts are insufficient to impair the ability to perform an EDS. It seems that chronic circadian disruption might be necessary to elicit impairments in the ability to perform EDSs, as indicated by the superior performance of non-phase-shifted rats compared to phase-shifted. The fact that chronic phase-shifts are able to produce impairments in behavioural flexibility is consistent with the literature examining how phase-shifting impairs immediate function (Antoniadis et al., 2000; Devan et al., 2001). However, it is possible that chronic circadian disruption is altering the function of striatum, not PFC. In other words, the impairments on EDS performance are emerging from shortcomings in the system underlying formation of the association. This possibility was largely eliminated by the re-establishment of asymptotic performance levels prior to EDS training. Novel findings reported here include the observation that the impairments are not transient in that they extend past circadian reentrainment and the observed sex differences on each learning paradigm.

The observation that impairments persist after daily activity rhythm stabilization is also an extremely important observation. The implications of long term impairment following circadian disruption has important implications for individuals who are

employed in positions that produce circadian disruption such as pilots, paramedics, nurses, doctors, or factory workers. Furthermore, these results indicate that in addition to the effects of phase-shifting on learning reported elsewhere (Craig & McDonald, 2008; Devan et al., 2000), phase-shifting produces impairments in retention of previously learned associations within both HPC and striatum. It could be the case that potentially fatal errors by pilots, doctors, or nurses are related to the chronic circadian disruption many experience. The present findings indicate that scheduling practice throughout the transportation and health care industries need to be examined.

Histology

The prediction that circadian disruption would be associated with changes in cellular metabolism (see Chapter One) was assessed by examining a highly plastic aspect of neuroanatomy in HPC and PFC: mitochondrial morphology. Regarding the observations of mitochondrial morphology, it may be the case that the interval between the circadian disruption and tissue collection was too great given the plasticity of mitochondrial density. Consequently, there was no effect of phase-shift observed for mitochondrial density. Perhaps examining mitochondrial morphology immediately following circadian disruption would increase the chances of observing significant differences on this measure.

Alternative Hypotheses

Stress. Stress is likely contributing to the observed effects. The release of glucocorticoids follows a relatively steady circadian rhythm (Chrousos, 1998). The rhythmicity exhibited by glucocorticoid expression is both up- and down-stream of the

SCN. Glucocorticoids are a diffusible signal by which the SCN communicates with other neural regions (Dickmeis, 2009). However, glucocorticoids are not the only means of communication, both diffusible and direct (e.gs., androgen hormones, insulin, etc). Thusly, it is likely that glucocorticoids are acting in concert with other neural pathways to produce the observed learning and memory effects associated with phase-shifting. So, although phase-shifting may lead to increases in the expression of glucocorticoids, it is unlikely the sole source of the observed effects.

Sleep. Previous studies have reported similar behavioural effects following periods of sleep disruption (Witt et al., 2010). Therefore, it is parsimonious to assume that the behavioral impairments observed here are related to sleep. However, previous reports indicate that although phase-shifting disrupts sleep bout onset and duration, it does not affect sleep phase ratios (e.g., REM, non-REM; Uschakov et al., 2007). In fact, even complete ablation of SCN does not impact these proportions in a meaningful fashion. The SCN is responsible for the onset and duration of sleep, other nuclei are responsible for sleep rhythms. Sleep rhythms, not to be confused with circadian rhythms, are located mainly in reticulo-pontine regions and hypothalamus (Pace-Schott & Hobson, 2002). Thus, although the timing of sleep is affected by phase-shifting, the ratio and amount of the components of sleep remain relatively intact (Pace-Schott & Hobson, 2002). Therefore, the observed behavioural impairments should not be attributed to sleep because the components of sleep (i.e., sleep phases) are largely unaffected. In addition, behavioural testing following the phase-shift did not begin until circadian rhythms had re-stabilized. Therefore, although unquantified, it is unlikely that sleep impairments are driving the behavioural effects.

Conclusion and Future Directions

Phase-shifting disrupts circadian rhythm generation (Craig & McDonald, 2008; Devan et al., 2000). Although some support exists, the identification of cognitive impairments associated with this arrhythmicity are less frequent (Antoniadis & McDonald, 2000), particularly the relationship between the underlying physiology and behavioural impairments. Based on the finding that running wheels appear to ameliorate the behavioural effect, it could be the case that phase-shifting is impacting apoptosis and neurogenesis. Apoptosis could be assessed at various time points across the cycle with markers for Caspase-3 and -9 (Portier et al., 2006). Furthermore, neurogenesis following the phase-shift could be assessed using markers for BDNF or Neu-N (for which a sex difference has been observed).

Regardless of the lack of histological correlates, further investigation is warranted by the observed behavioural deficits and conjectured physiological sources. Further research should not only examine the effects of phase-shifting, it should imbed the study of phase-shifting within sex and exercise to ascertain information not only on phase-shifts and the associated behavioural impairments, but on cellular processes such as neurogenesis, apoptosis, and survival as well. The finding that the effects of phase-shifting are largely ameliorated by exercise and sex make phase-shifting a potential model for conducting research on cellular phenomena such as neurogenesis without an express interest in phase-shifting.

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TABLES

Table 2.1. Acute phase-shift schedule.

DAY	ON	OFF	ON
1	4:30 AM (0430)	4:30 PM (1630)	
2	1:30 AM (0130)	1:30 PM (1330)	10:30 PM (2230)
3		10:30 AM (1030)	7:30 PM (1930)
4		7:30 AM (0730)	4:30 PM (1630)
5		4:30 AM (0430)	1:30 PM (1330)
6		1:30 AM (0130)	
7	1:30 PM (1330)	1:30 AM (0130)	

Table 3.1 Chronic phase-shift schedule.

Day	ON	OFF	ON	OFF
1	7:30 AM (07:30)	7:30 PM (19:30)		
2	4:30 AM (04:30)	4:30 PM (16:30)		
3	1:30 AM (01:30)	1:30 PM (13:30)	10:30 PM (22:30)	
4		10:30 AM (10:30)	7:30 PM (19:30)	
5		7:30 AM (07:30)	4:30 PM (16:30)	
6		4:30 AM (04:30)	1:30 PM (13:30)	
7 - 15		1:30 AM (01:30)	1:30 PM (13:30)	
16/17		1:30 AM (01:30)	1:30 PM (13:30)	10:30 PM (22:30)
18	10:30 AM (10:30)	7:30 PM (19:30)		
19	7:30 AM (07:30)	4:30 PM (16:30)		
20	4:30 AM (04:30)	1:30 PM (13:30)		
21	1:30 AM (01:30)	10:30 AM (10:30)	10:30 PM (22:30)	
22		7:30 AM (07:30)	7:30 PM (19:30)	
23-33		4:30 AM (04:30)	4:30 PM (16:30)	
34		4:30 AM (04:30)	1:30 PM (13:30)	
35		1:30 AM (01:30)		
36	10:30 AM (10:30)	10:30 PM (22:30)		
37	7:30 AM (07:30)	7:30 PM (19:30)		
38	4:30 AM (04:30)	4:30 PM (16:30)		
39	1:30 AM (01:30)	1:30 PM (13:30)	10:30 PM (22:30)	
40		10:30 AM (10:30)	7:30 PM (19:30)	
41-48		7:30 AM (07:30)	7:30 PM (19:30)	
49		4:30 AM (04:30)	4:30 PM (16:30)	
50		1:30 AM (01:30)	1:30 PM (13:30)	10:30 PM (22:30)
51			10:30 AM (10:30)	7:30 PM (19:30)
52			7:30 AM (07:30)	4:30 PM (16:30)
53			4:30 AM (04:30)	1:30 PM (13:30)
54			1:30 AM (01:30)	10:30 AM (10:30)
55+			10:30 PM (22:30)	10:30 AM (10:30)

FIGURES

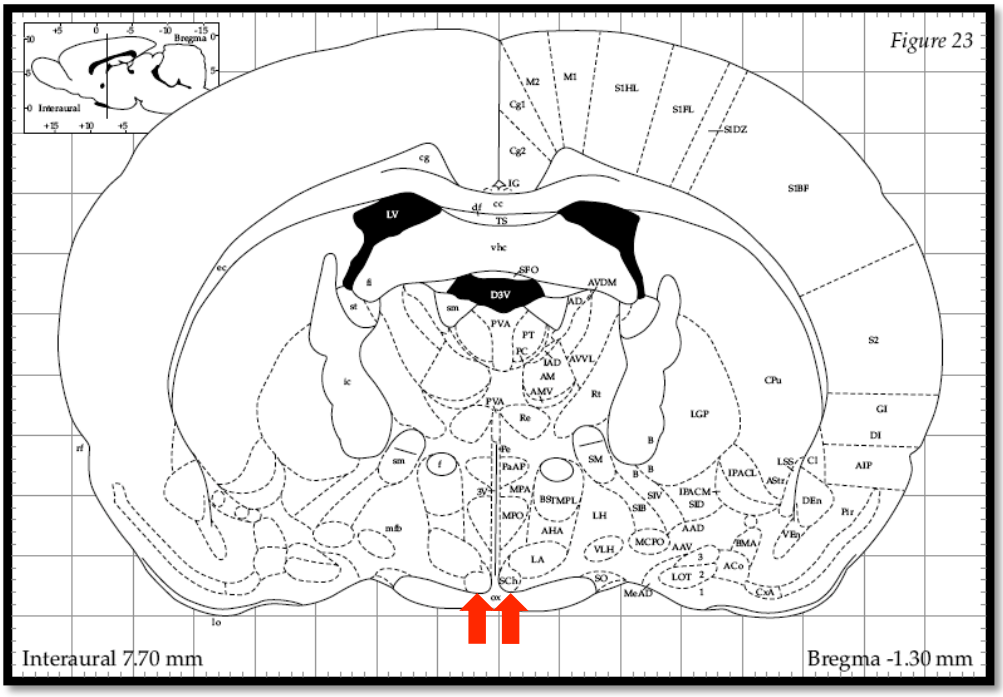

Figure 1.1 Schematic representation of the Suprachiasmatic Nucleus (SCN) of the rat.

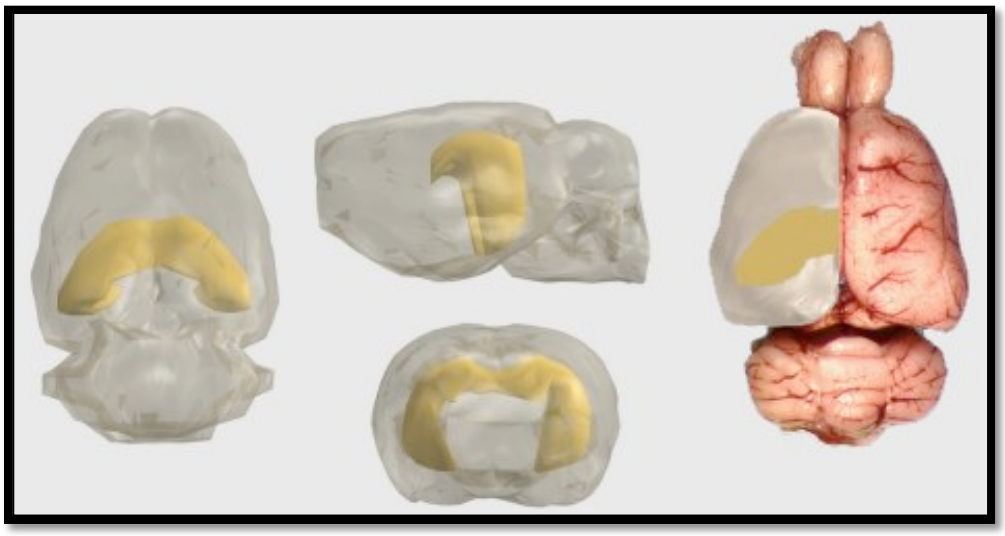

Figure 1.2 Schematic representation of the Hippocampus (HPC) of the rat retreived from http://synapses.clm.utexas.edu/anatomy/hippo/hippo.stm

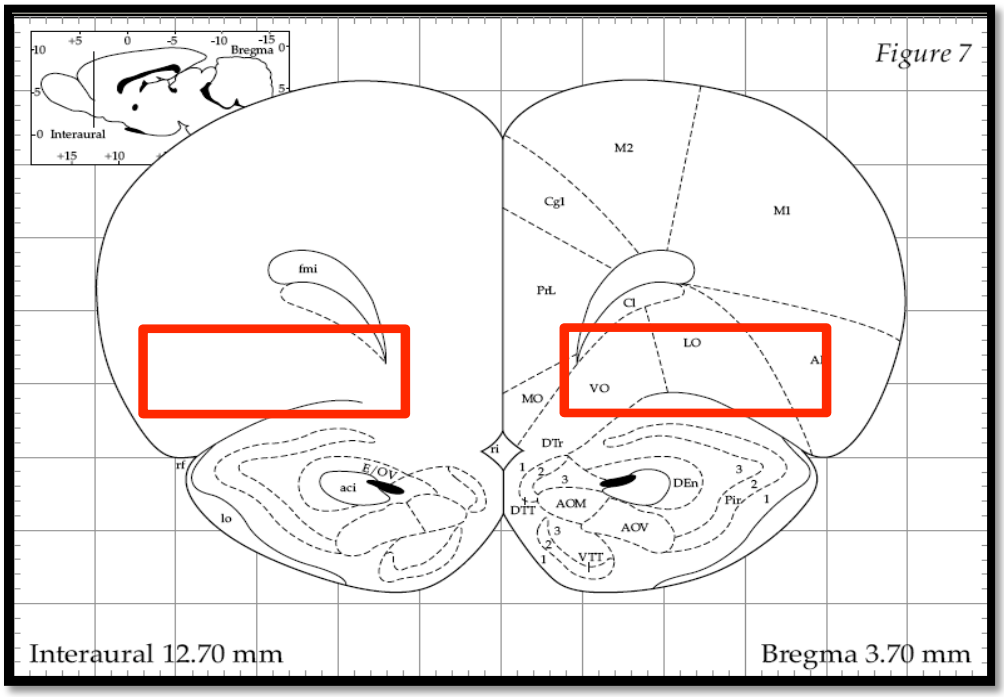

Figure 1.3 Schematic representation of the Prefrontal Cortex (PFC) of the rat. The areas outlined in red represent Orbital Prefrontal Cortex (OPFC).

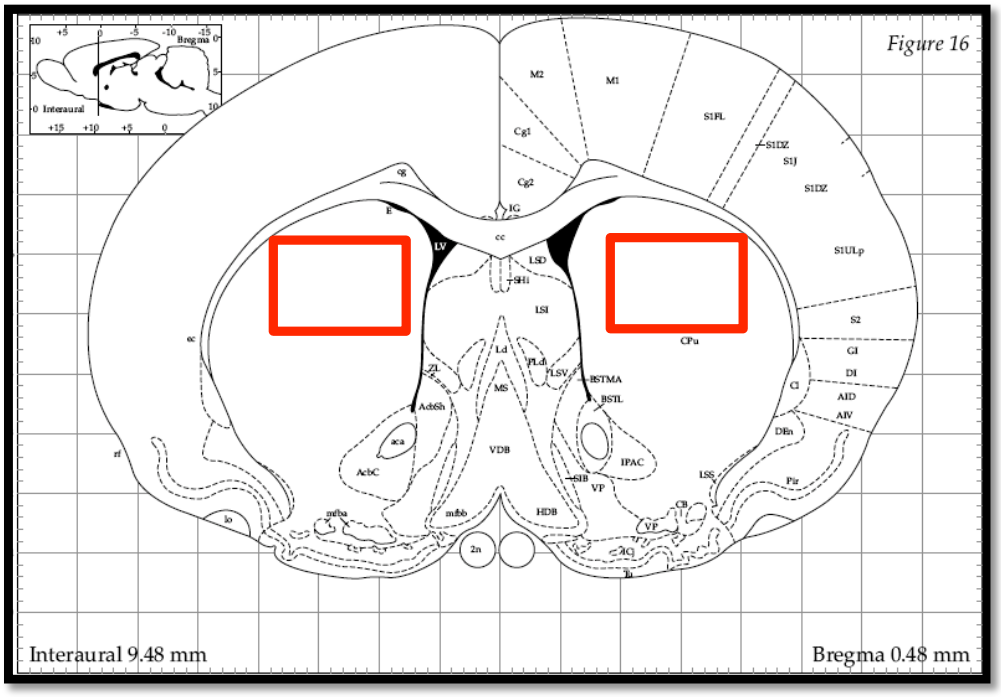

Figure 1.4

Schematic representation of a rat brain with the Dorsal Striatum (DS) outlined in red.

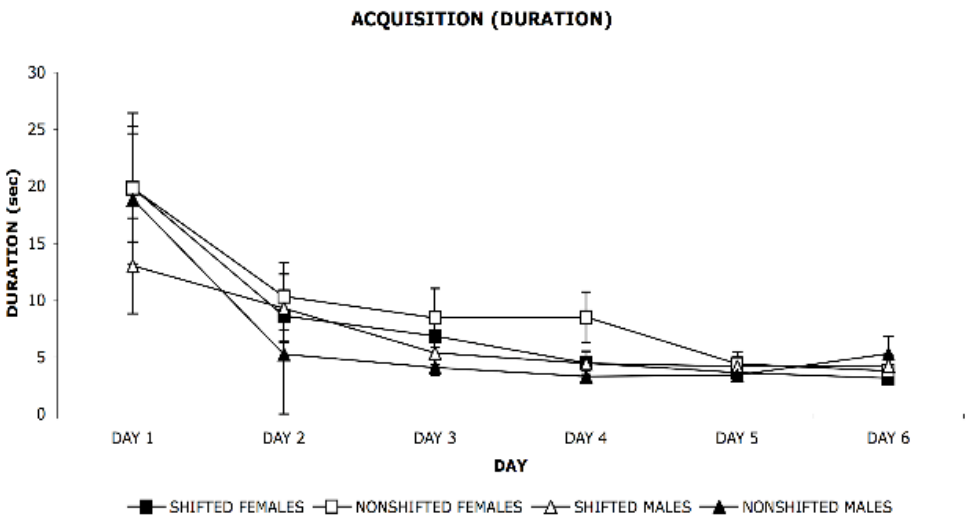

Figure 2.1a Experiment One. Trial duration during acquisition on MWT grouped by sex and phase-shift.

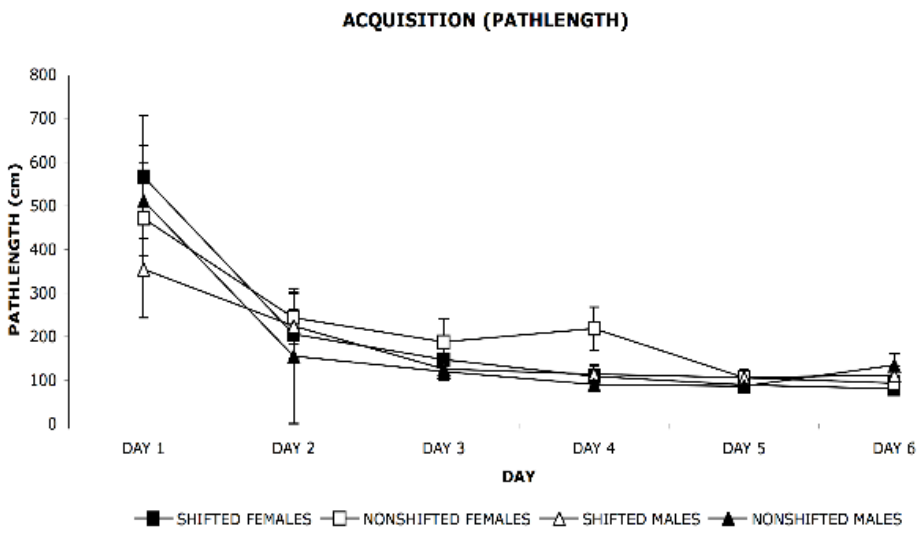

Figure 2.1b Experiment One. Pathlength during acquisition of MWT grouped by sex and phase-shift.

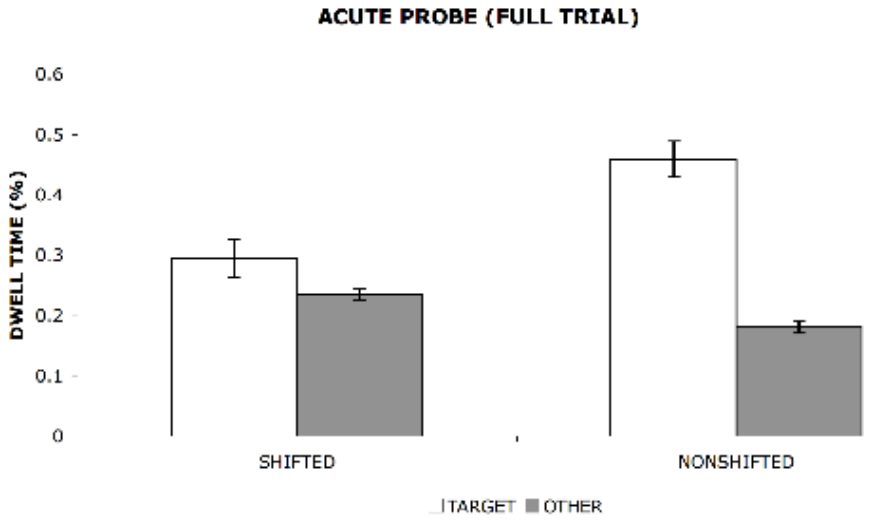

Figure 2.2a Experiment One. Retention of the MWT probe location for the full trial duration grouped by acute phase-shift condition.

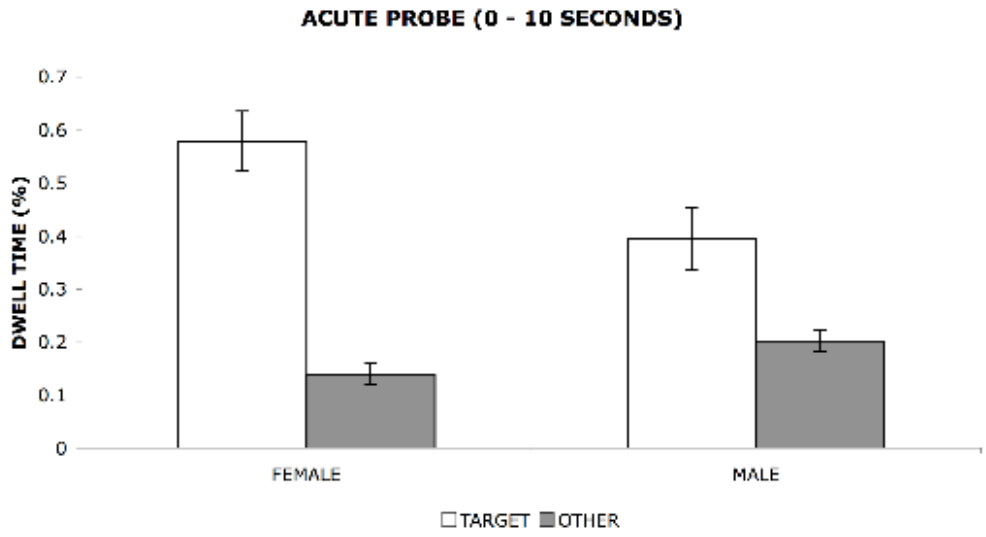

Figure 2.2b Experiment One. Acute phase-shift retention of the MWT probe location during the first 10 seconds of the probe trial grouped by sex.

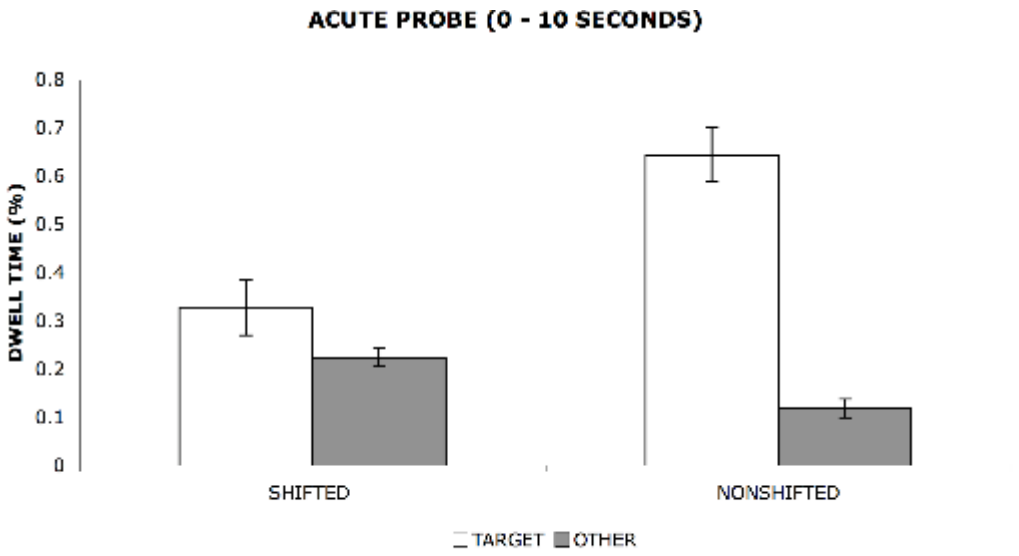

Figure 2.2c Experiment One. Retention of the MWT probe location during the first 10 seconds of the probe trial grouped by phase-shift.

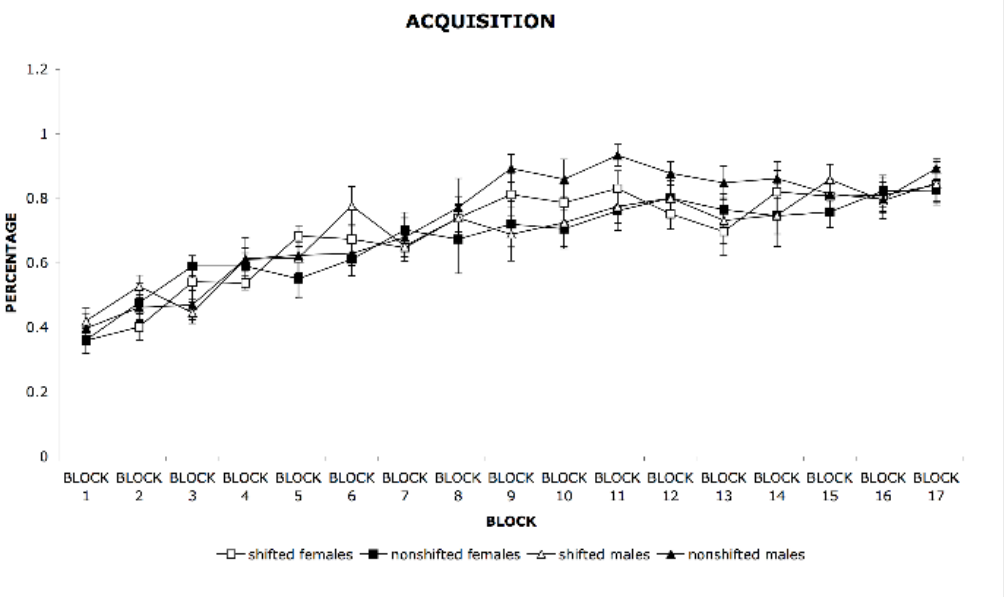

Figure 2.3a Experiment One. Acquisition of the visual discrimination task developed for 8-arm radial maze depicting overall performance grouped by sex and phase-shift.

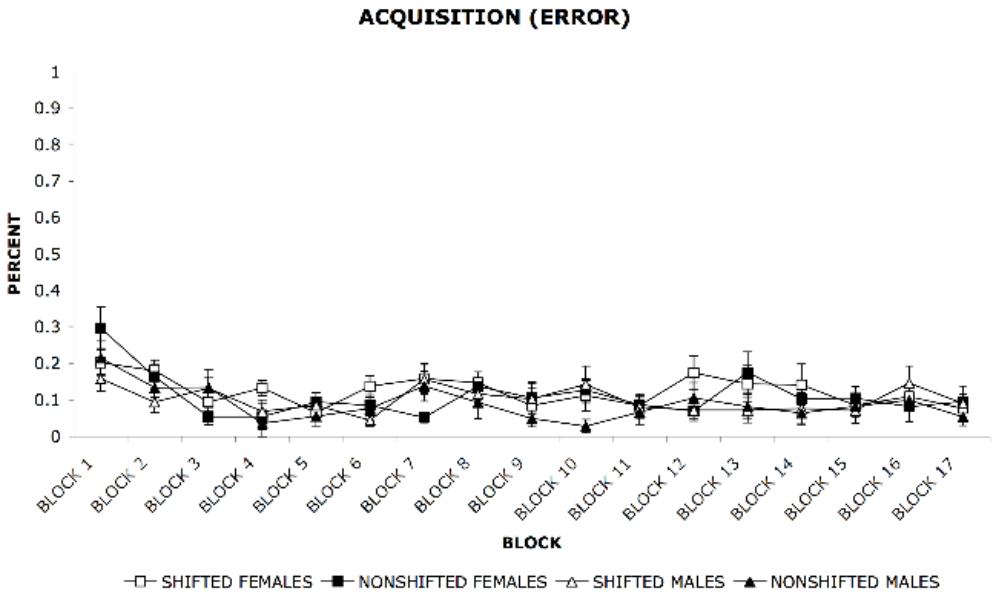

Figure 2.3b Experiment One. Acquisition of the visual discrimination task developed for the 8-arm radial maze depicting percentage of errors grouped by sex and phase-shift.

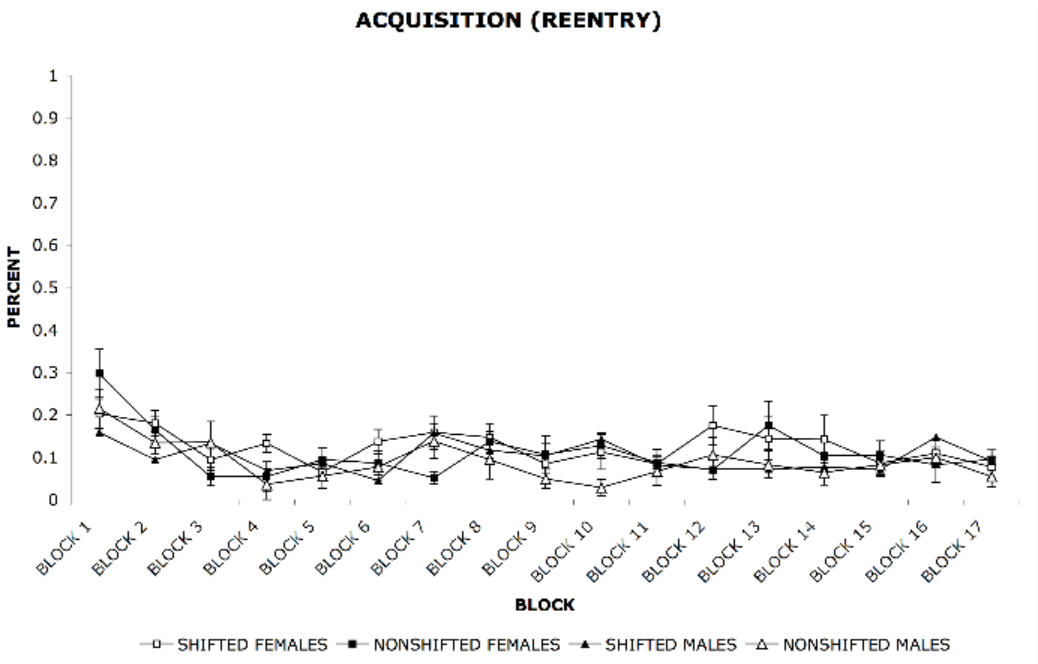

Figure 2.3c Experiment One. Acquisition on the visual discrimination task developed for the 8-arm radial maze depicting percentage of re-entries grouped by sex and phase-shift.

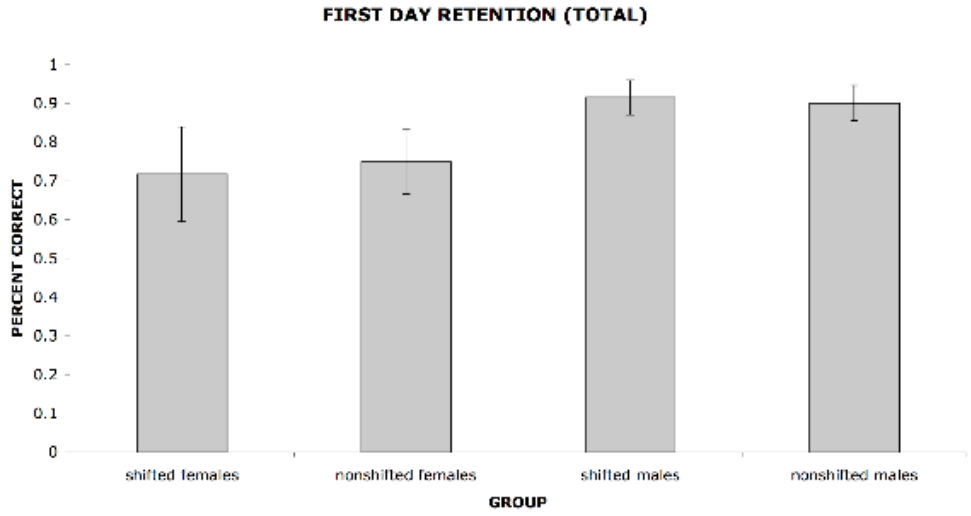

Figure 2.4a Experiment One. Overall performance on the first day of retention testing on the visual discrimination task developed for the 8-arm radial maze grouped by sex and phase-shift.

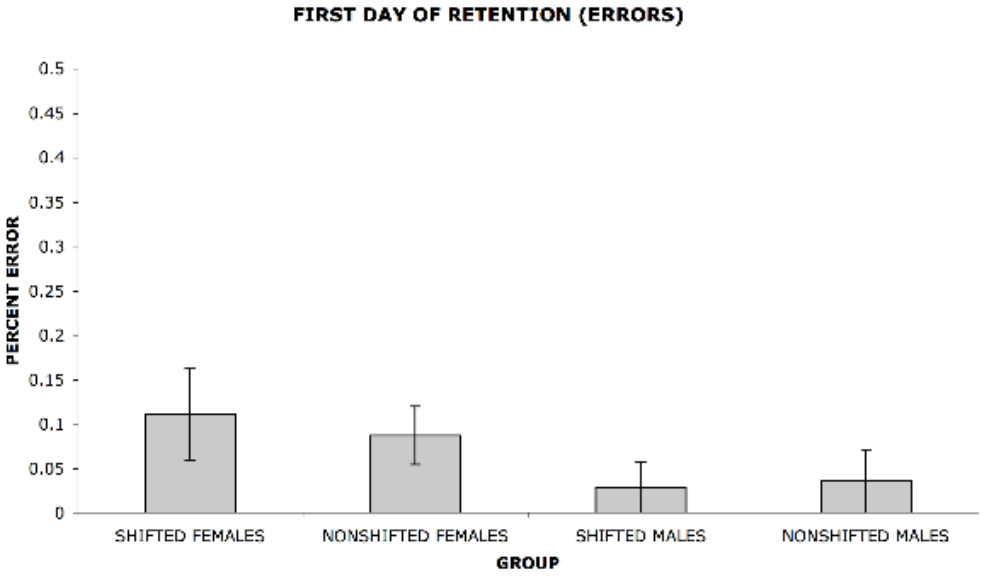

Figure 2.4b Experiment One. The percentage of erroneous entries on the first day of retention testing on the visual discrimination task developed for the 8-arm radial maze grouped by sex and phase-shift.

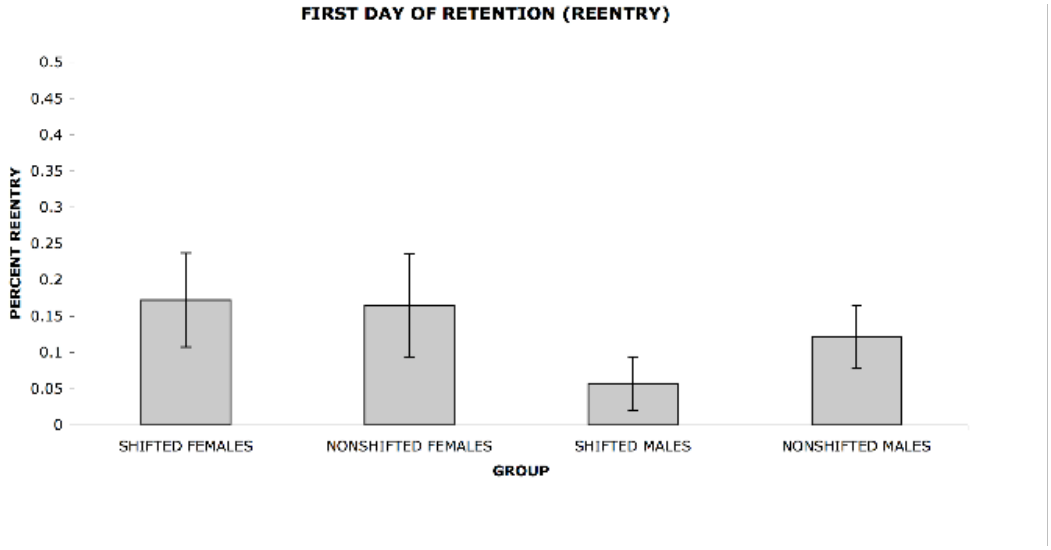

Figure 2.4c Experiment One. The percentage of re-entries on the first day of retention testing on the visual discrimination task developed for the 8-arm radial maze grouped by sex and phase-shift.

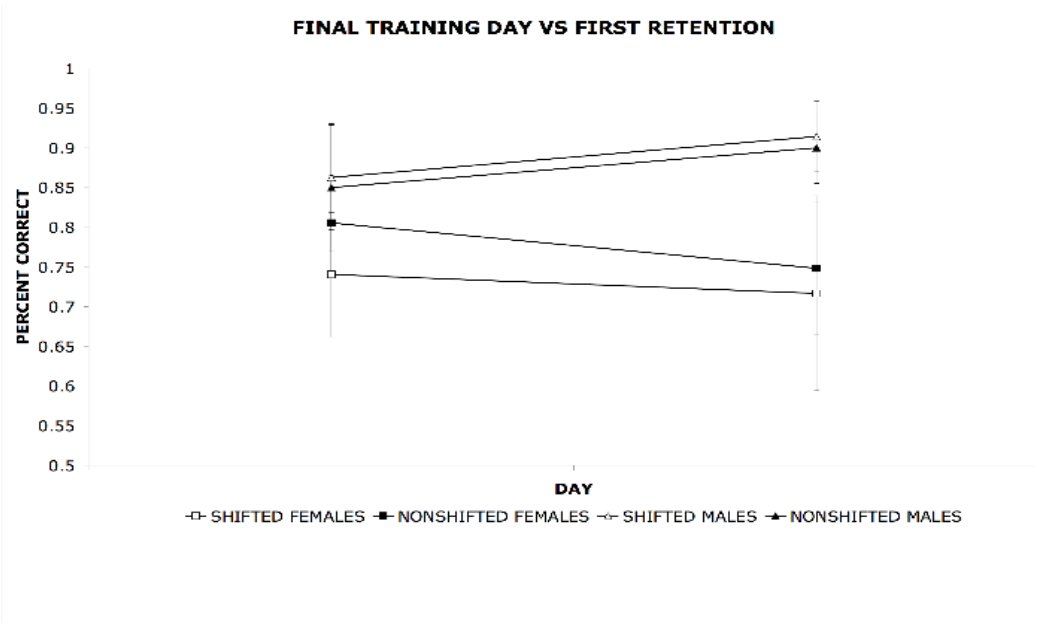

Figure 2.5 Experiment One. Comparison for overall performance between the final day of training to the first day of retention testing on the visual discrimination task following phase-shifting grouped by sex and phase-shift.

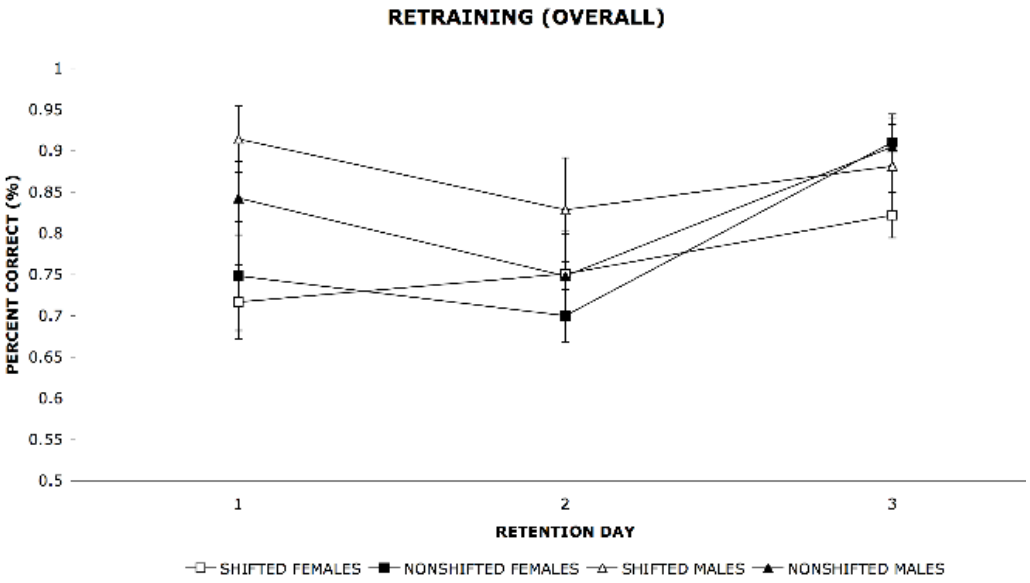

Figure 2.6a Experiment One. The reestablishment of asymptotic performance levels on the visual discrimination task for overall performance following phase-shifting grouped by sex and phase-shift.

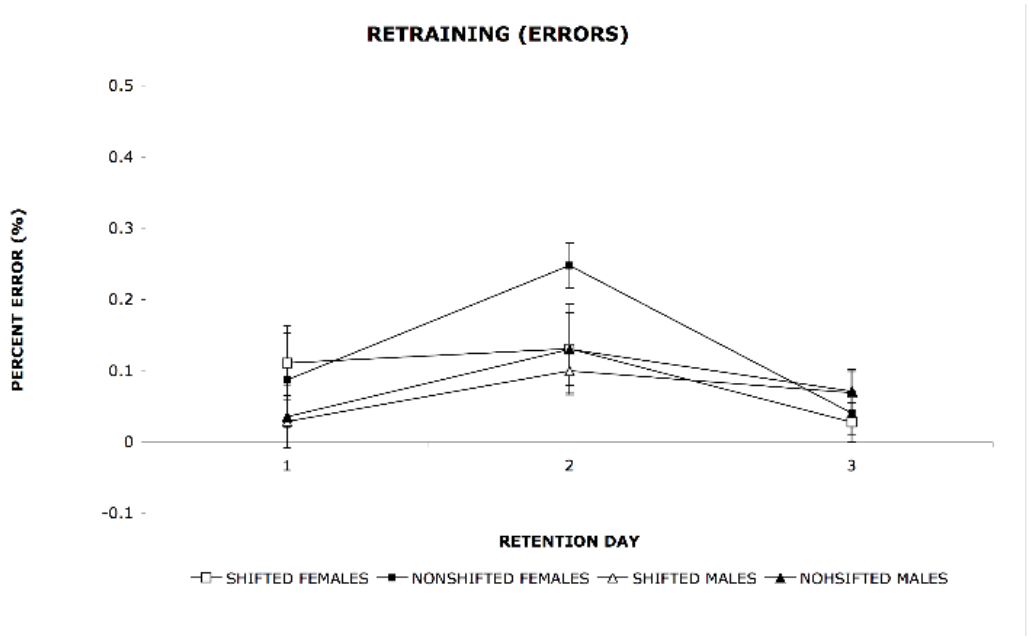

Figure 2.6b Experiment One. The reestablishment of asymptotic performance for percentage of errors made on the visual discrimination task following phase-shifting grouped by sex and phase-shift.

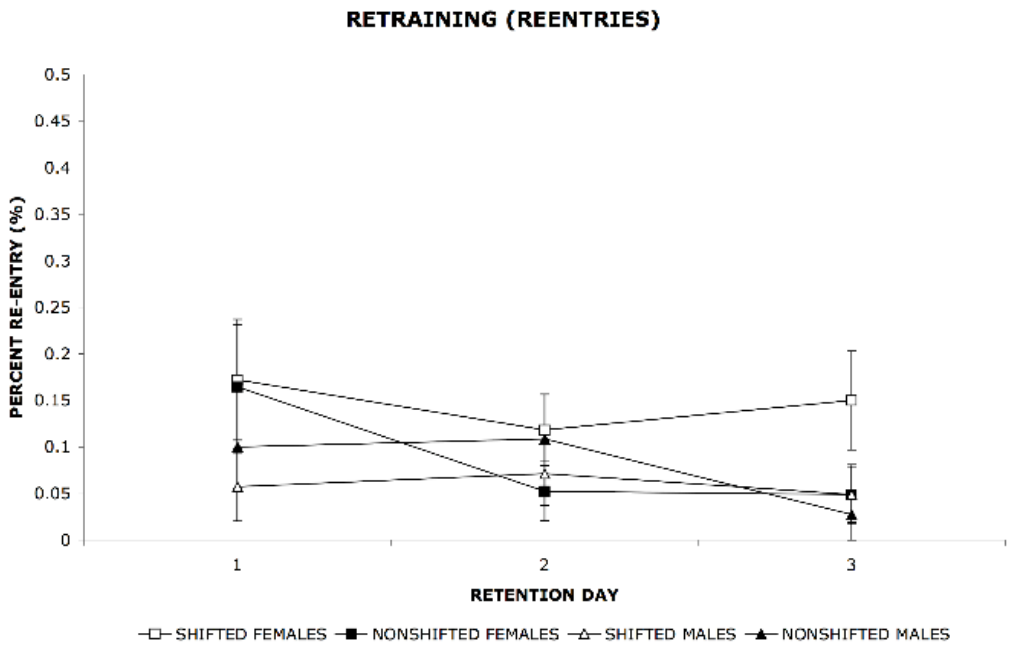

Figure 2.6c Experiment One. The reestablishment of asymptotic performance for percentage of reentries on the visual discrimination task following phase-shifting grouped by sex and phase-shift.

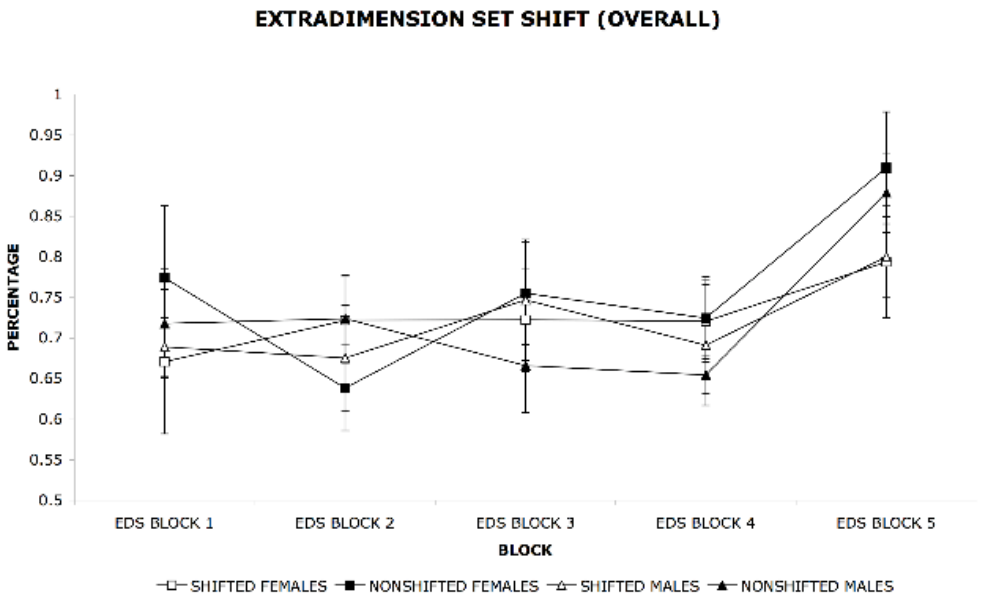

Figure 2.7a Experiment One. Overall performance of the extradimensional attentional set shift condition on the visual discrimination task grouped by sex and phase-shift.

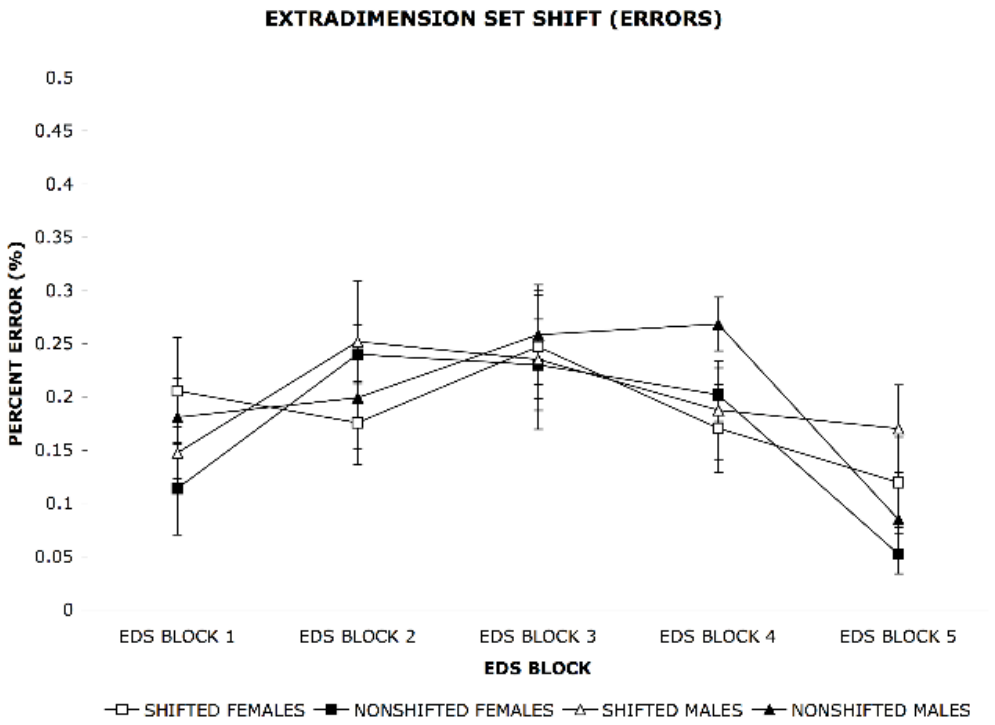

Figure 2.7b Experiment One. The percentage of errors committed during acquisition of the extradimensional attentional set shift condition of the visual discrimination task grouped by sex and phase-shift.

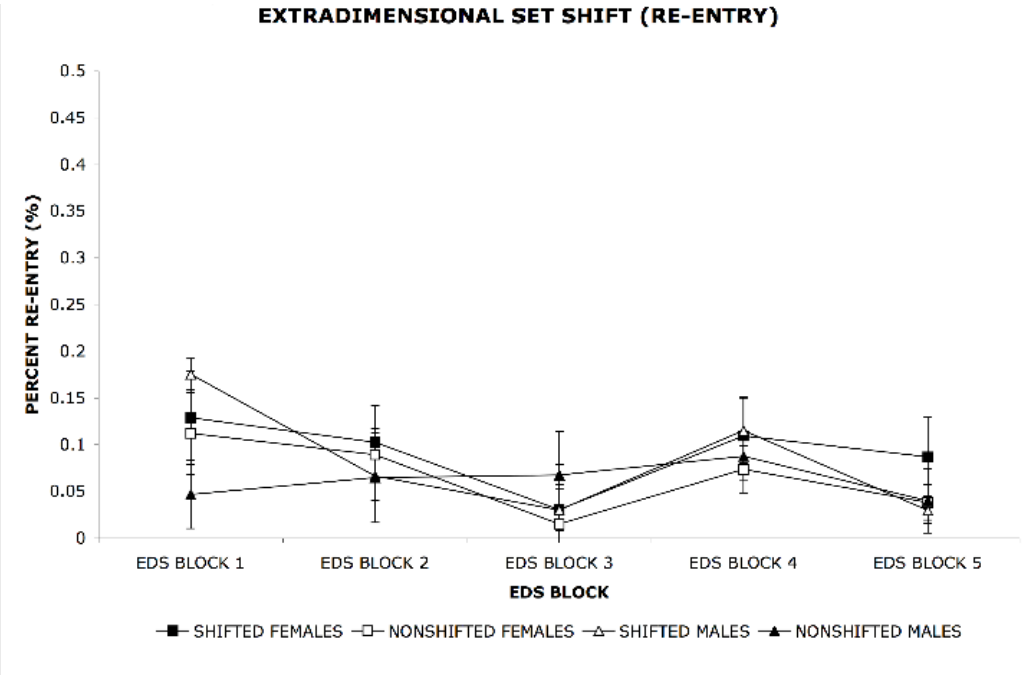

Figure 2.7c Experiment One. The percentage of re-entries committed during acquisition of the extradimensional attentional set shift condition of the visual discrimination task grouped by sex and phase-shift.

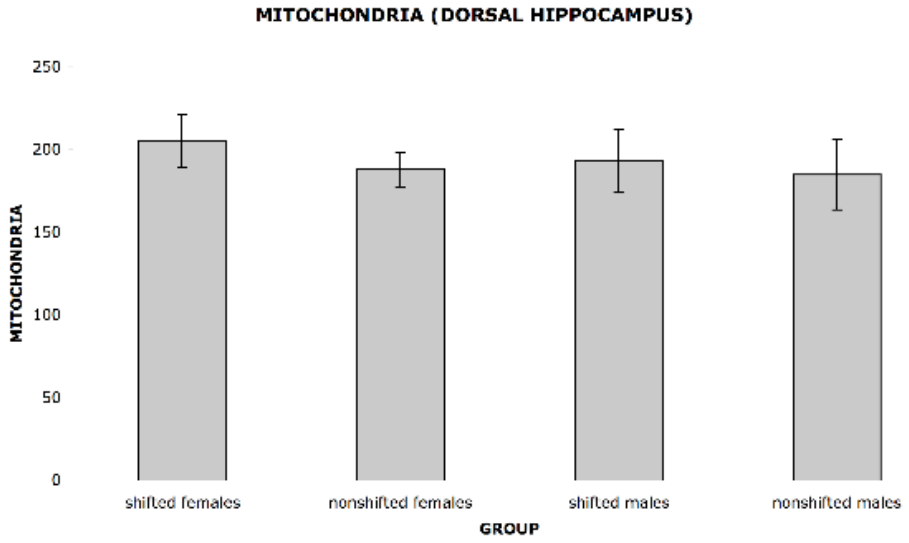

Figure 2.8a Experiment One. Assessment of mitochondrial density in dorsal hippocampus.

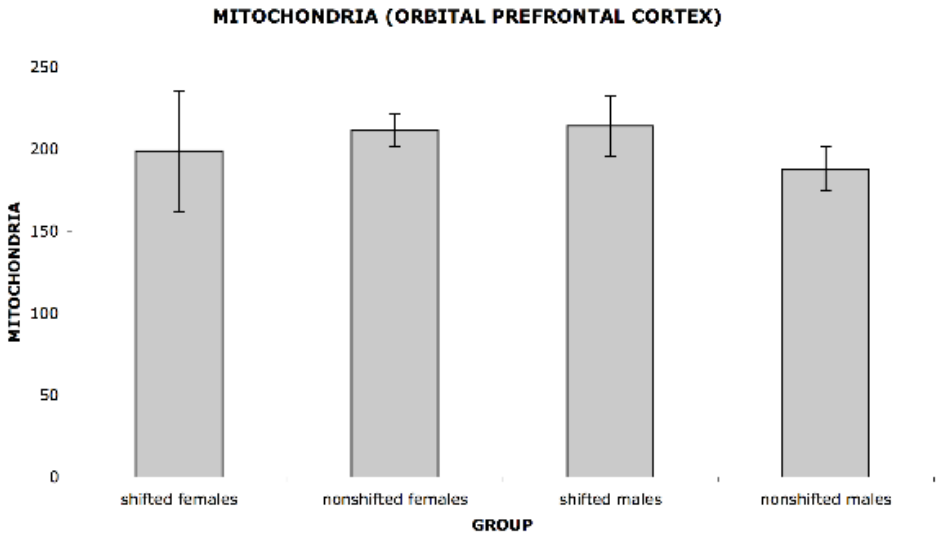

Figure 2.8b Experiment One. Assessment of mitochondrial density in orbital prefrontal cortex.

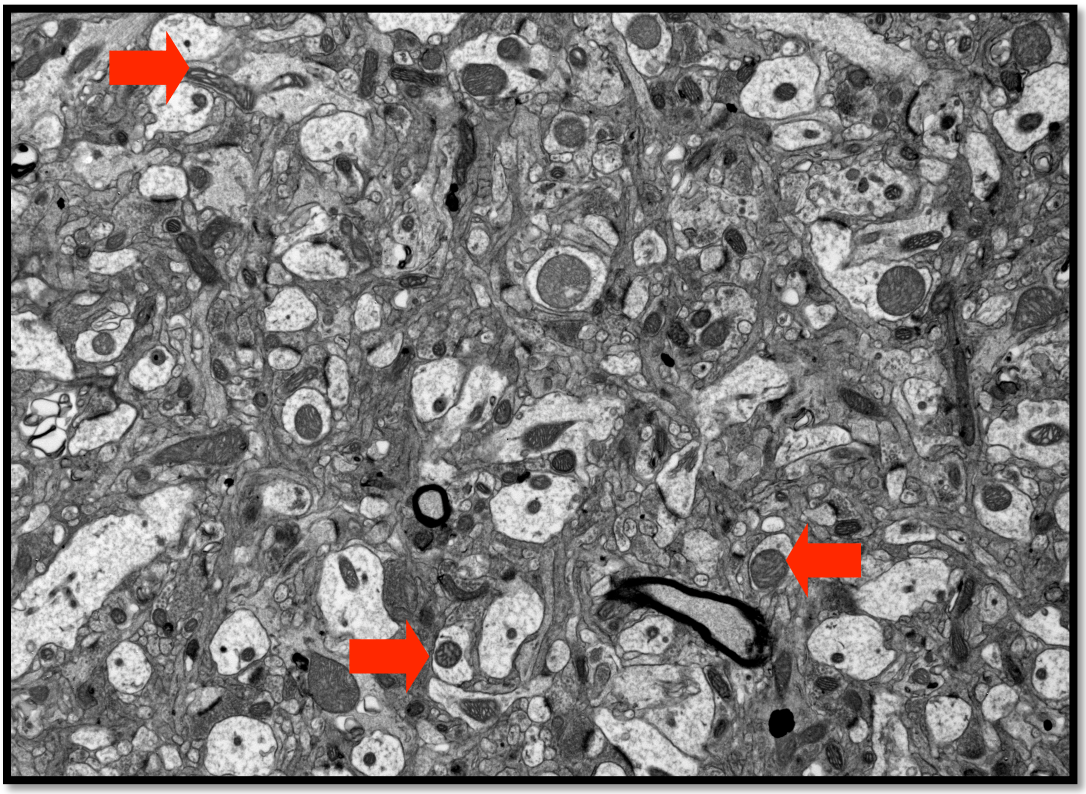

Figure 2.9 Experiment One. Photomicrograph depicting mitochondrial density in orbital prefrontal cortex. The red arrows indicate the location of three mitochondria. No significant differences were observed in association with sex or phase-shift.

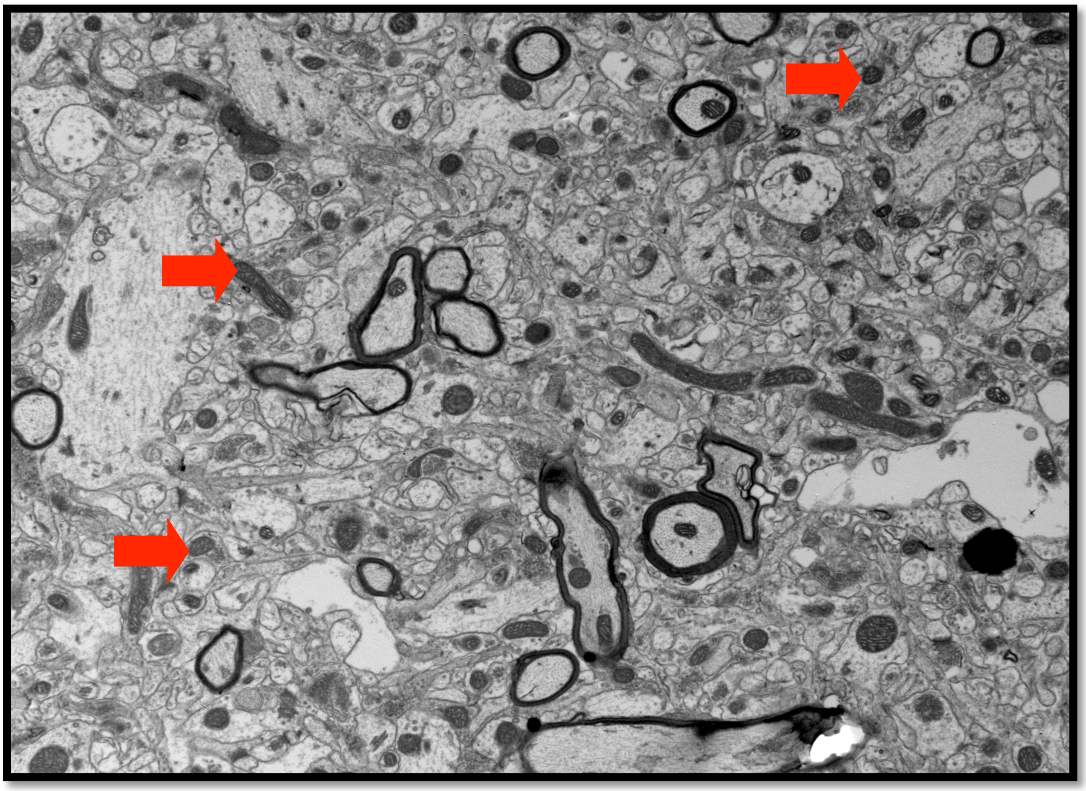

Figure 2.10 Experiment One. Photomicrograph depicting mitochondrial density in dorsal hippocampus. The red arrows indicate the location of three mitochondria. No significant differences were observed in association with sex or phase-shift.

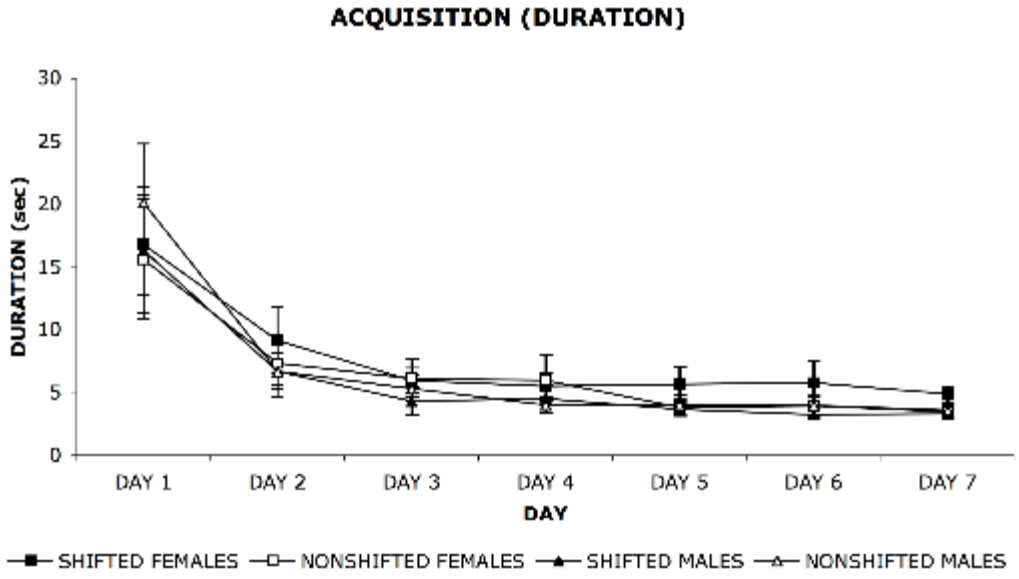

Figure 3.1a Experiment Two. Trial duration during acquisition of MWT grouped by sex and phase-shift.

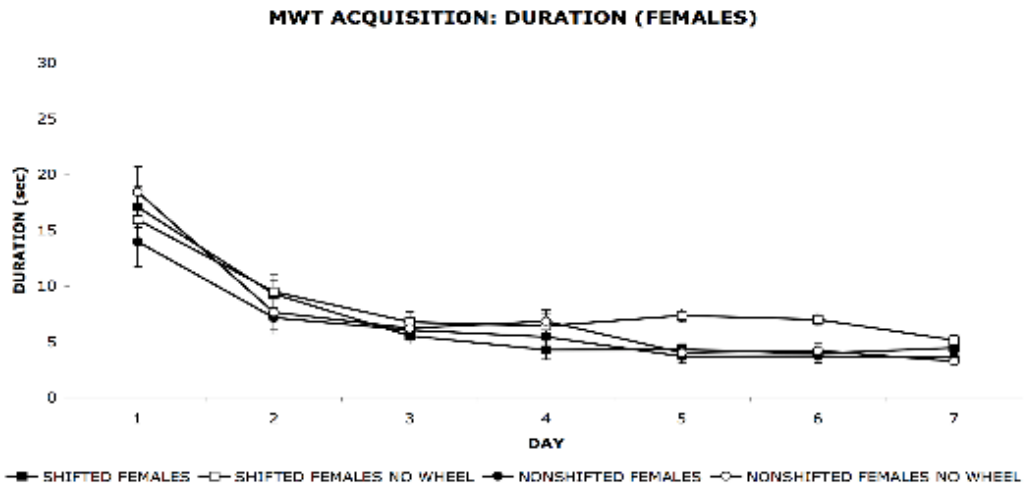

Figure 3.1b Experiment Two. Trial duration during acquisition of MWT for female rats only grouped by phase-shift and running wheel.

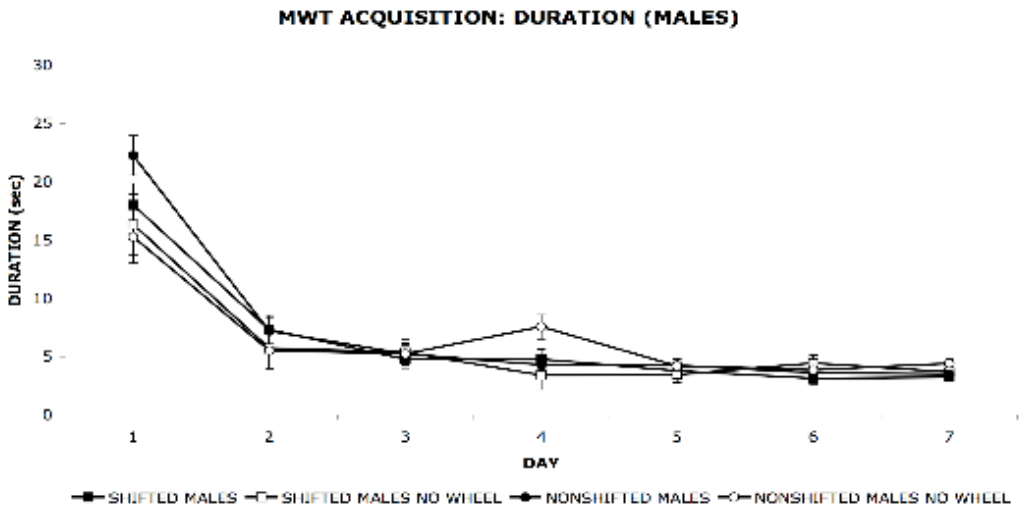

Figure 3.1c Experiment Two. Trial duration during acquisition of MWT for male rats only grouped by phase-shift and running wheel.

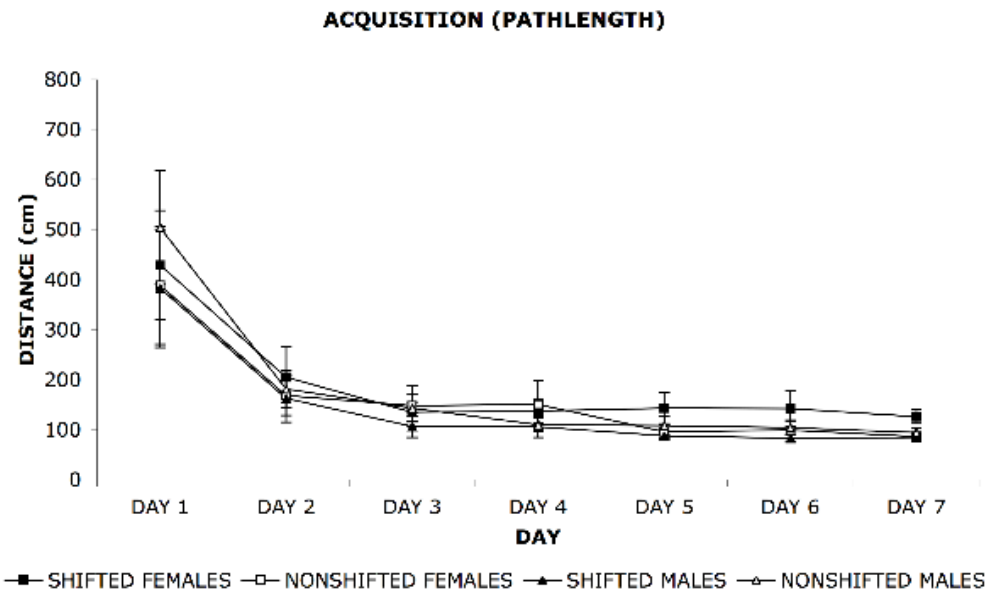

Figure 3.2a Experiment Two. Pathlength during acquisition of MWT grouped by sex and phase-shift.

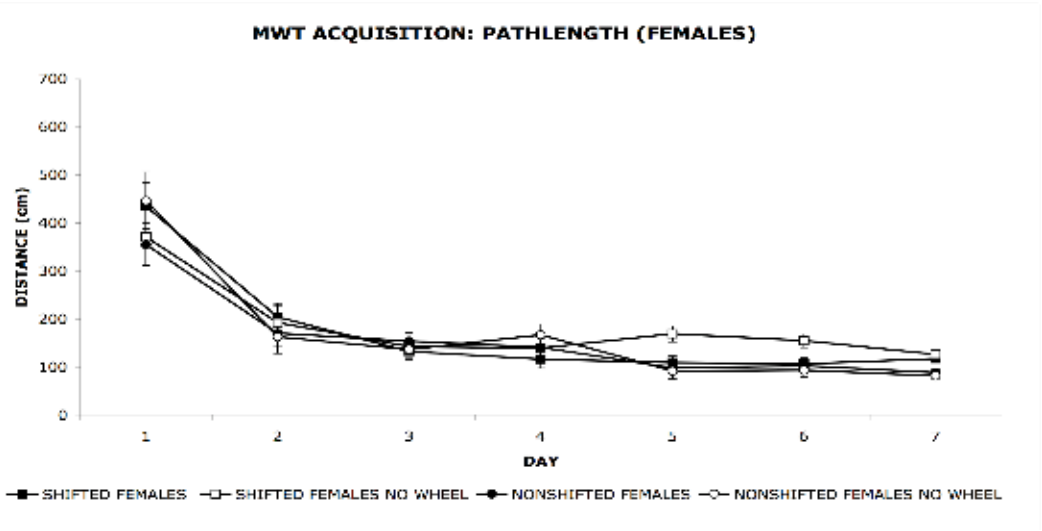

Figure 3.2b Experiment Two. Pathlength during acquisition of MWT for female rats only grouped by phase-shift and running wheel.

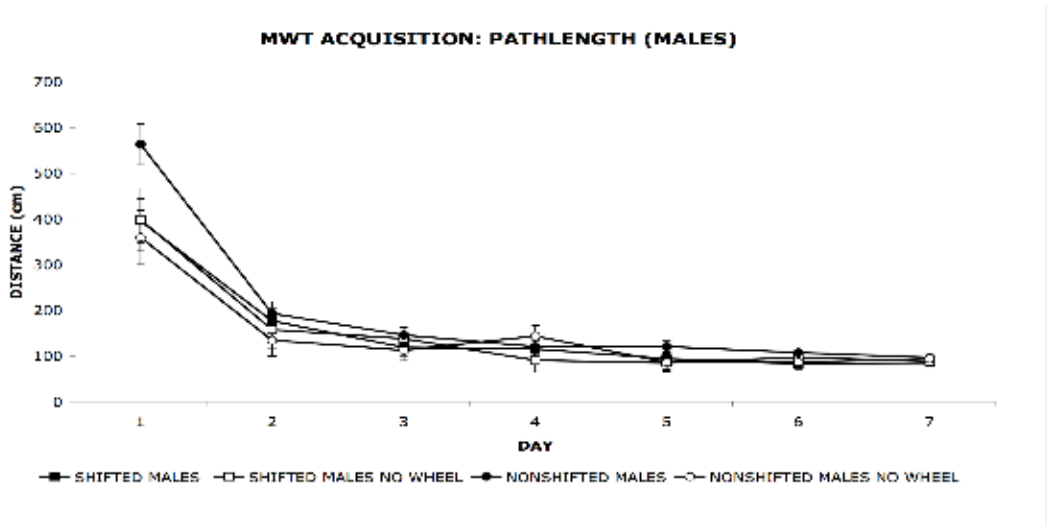

Figure 3.2c Experiment Two. Pathlength during acquisition of MWT for male rats only grouped by phase-shift and running wheel.

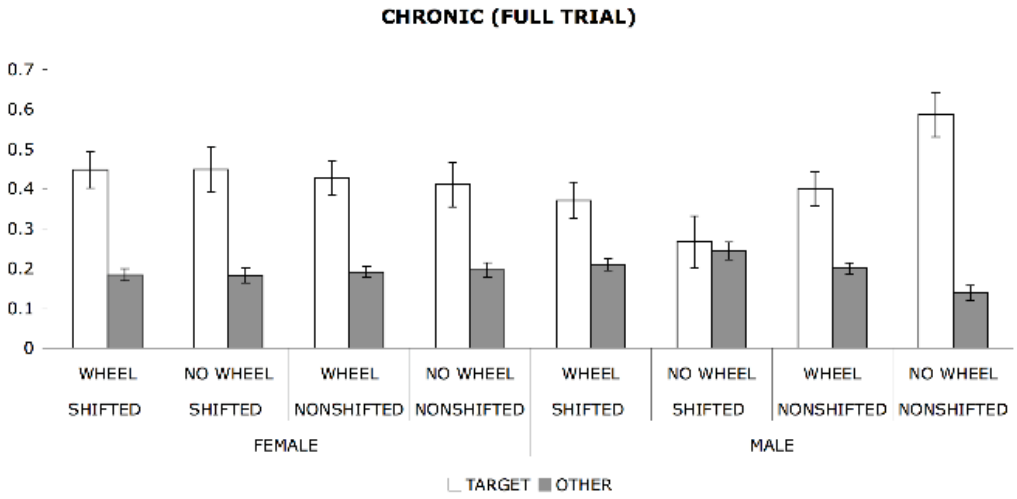

Figure 3.3a Experiment Two. Retention performance on the MWT probe test over the full trial duration grouped by sex, phase-shift, and running wheel condition.

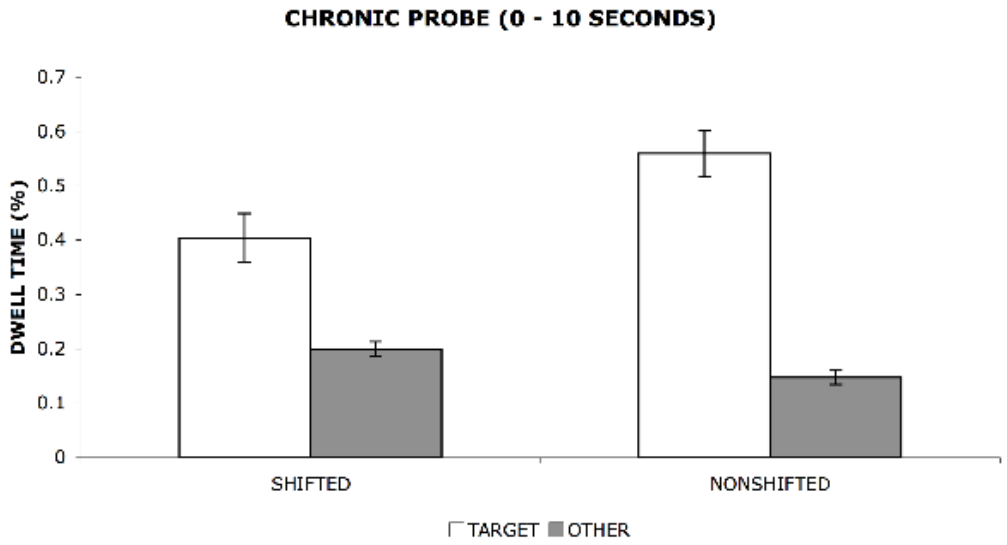

Figure 3.3b Experiment Two. Retention performance on the MWT probe test during the first 10 seconds of the trial grouped by phase-shift.

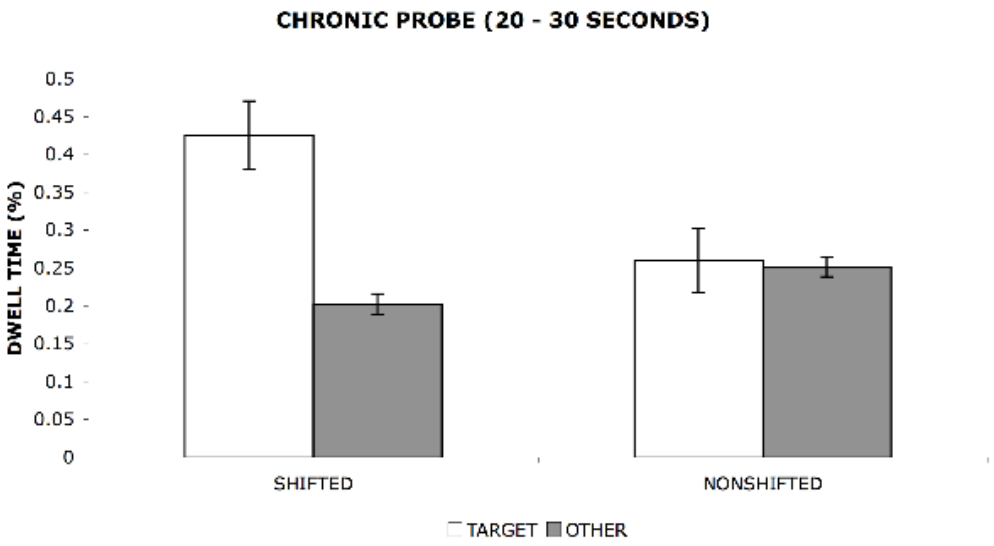

Figure 3.3c Experiment Two. Retention performance on the MWT probe test during the final 10 seconds of the trial grouped by phase-shift.

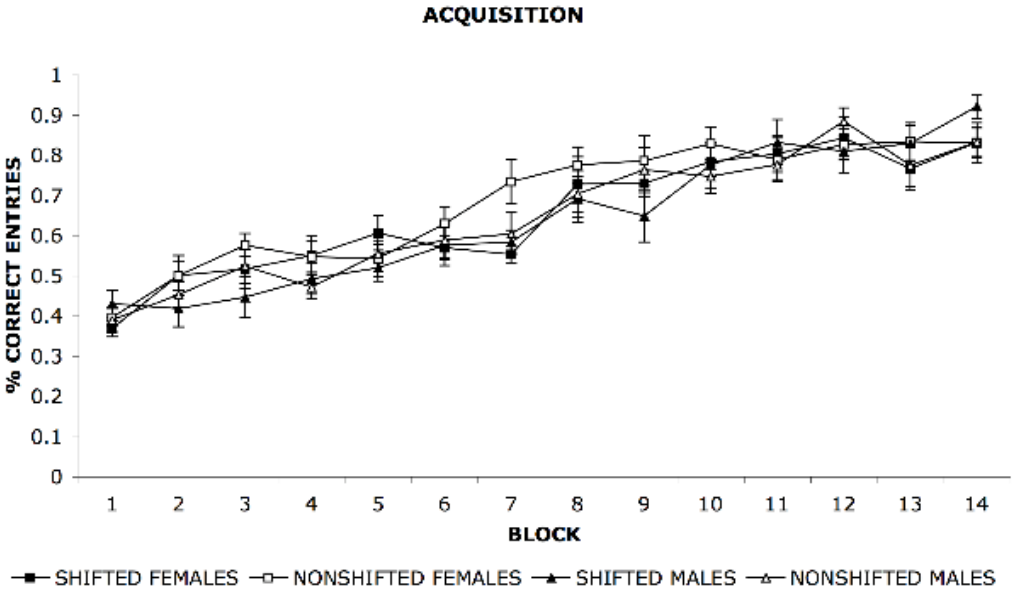

Figure 3.4a Experiment Two. Overall performance across acquisition training on the visual discrimination task developed for the 8-arm radial maze grouped by phase-shift condition and sex.

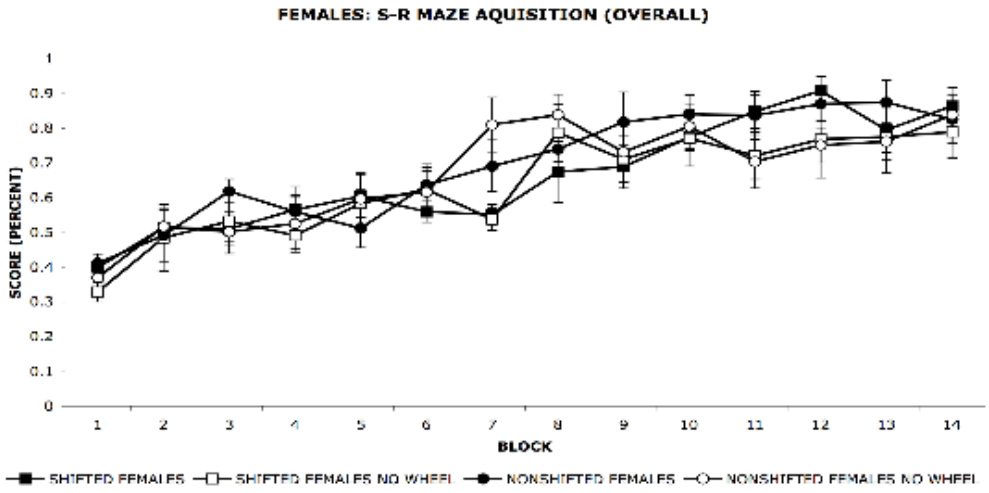

Figure 3.4b Experiment Two. Overall performance during acquisition training on the visual discrimination task developed for 8-arm radial maze for female rats only grouped by phase-shift and running wheel.

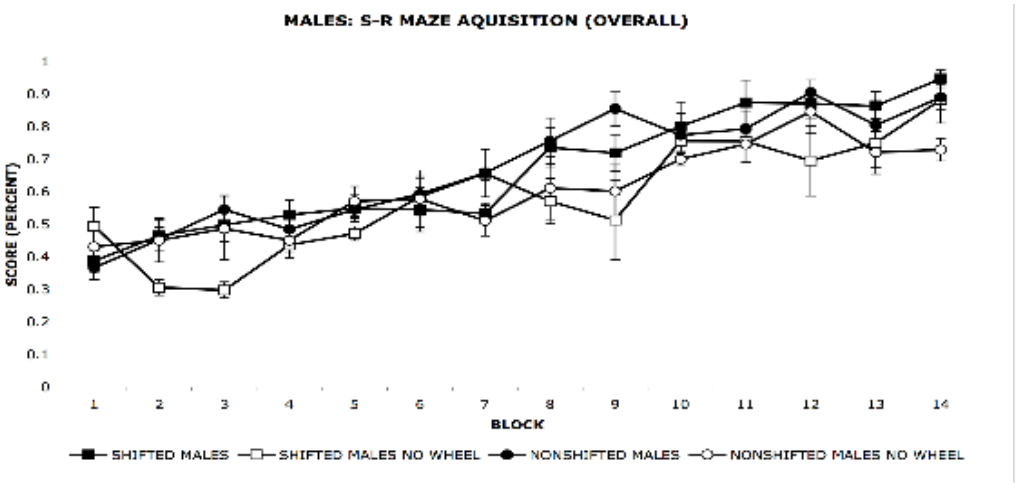

Figure 3.4c Experiment Two. Overall performance during acquisition training on the visual discrimination task developed for 8-arm radial maze for male rats only grouped by phase-shift and running wheel.

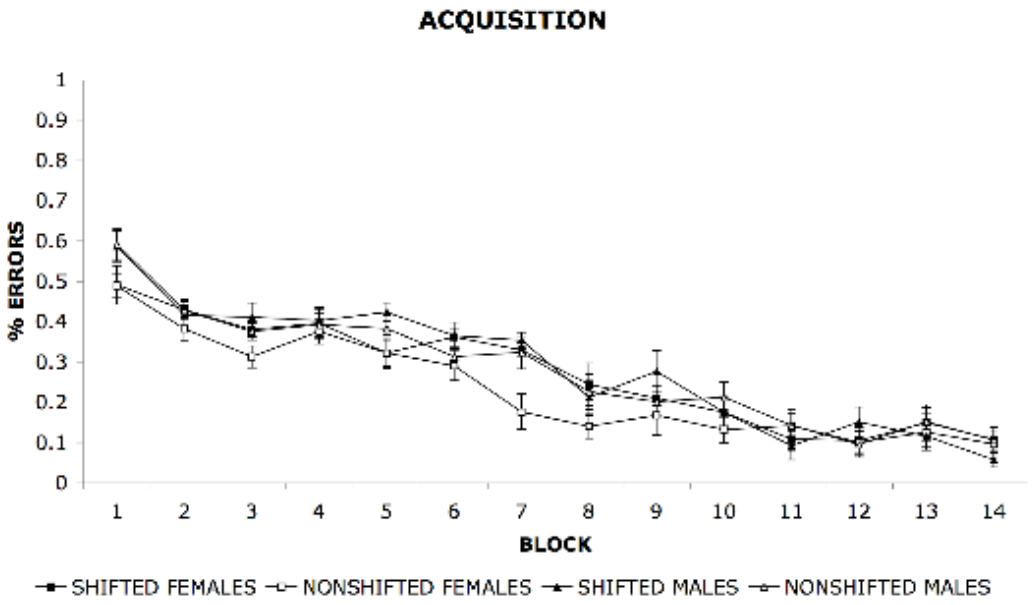

Figure 3.5a Experiment Two. The percentage of errors committed during acquisition training on the visual discrimination task developed for 8-arm radial maze grouped by sex and phase-shift.

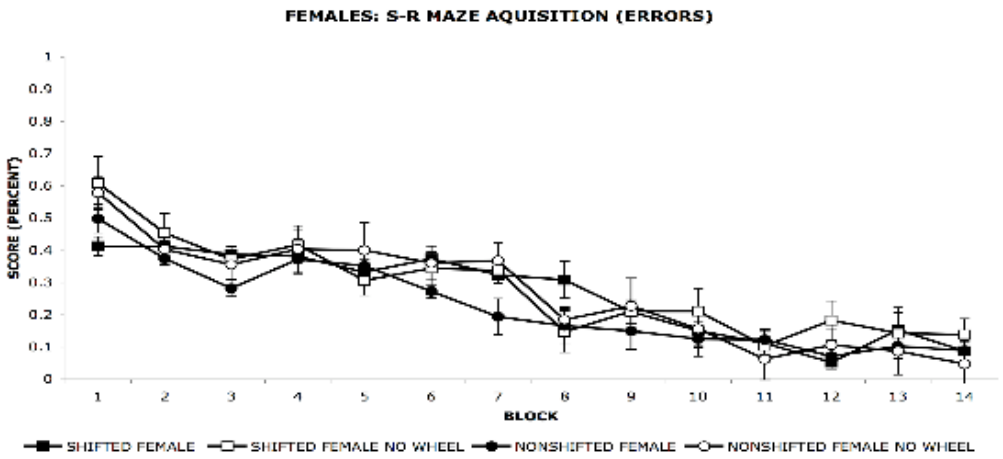

Figure 3.5b Experiment Two. The percentage of errors committed during acquisition training on the visual discrimination task developed for 8-arm radial maze for female rats only grouped by running wheel and phase-shift.

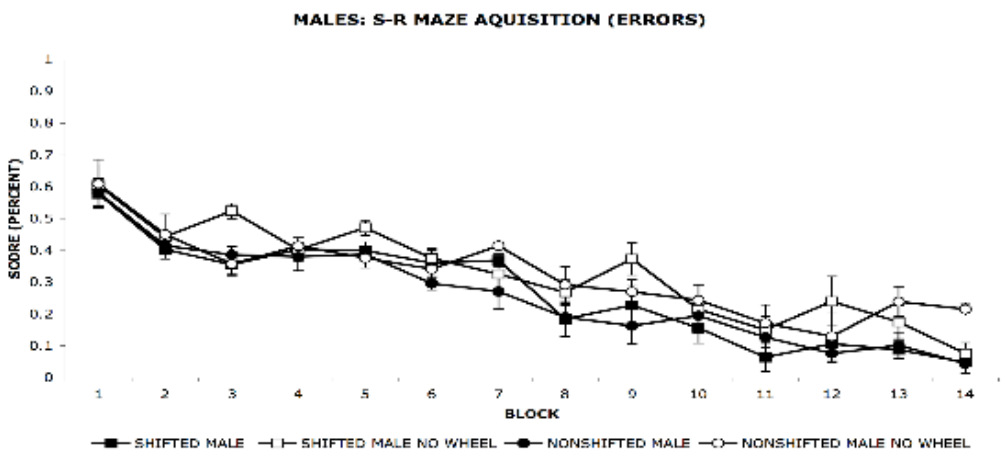

Figure 3.5c Experiment Two. The percentage of errors committed during acquisition training on the visual discrimination task developed for 8-arm radial maze for male rats only grouped by running wheel and phase-shift.

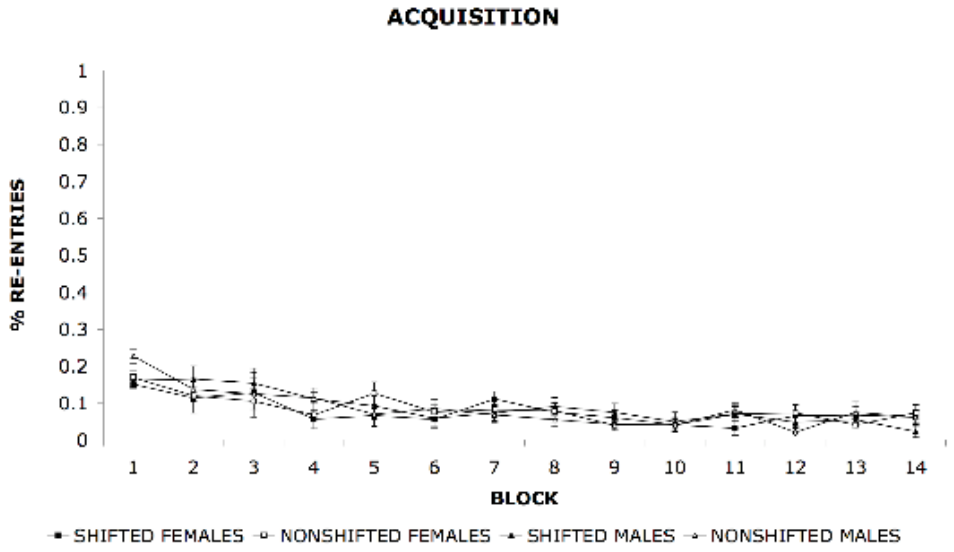

Figure 3.6a Experiment Two. The percentage of re-entries committed during acquisition training on the visual discrimination task developed for 8-arm radial maze grouped by sex and phase-shift.

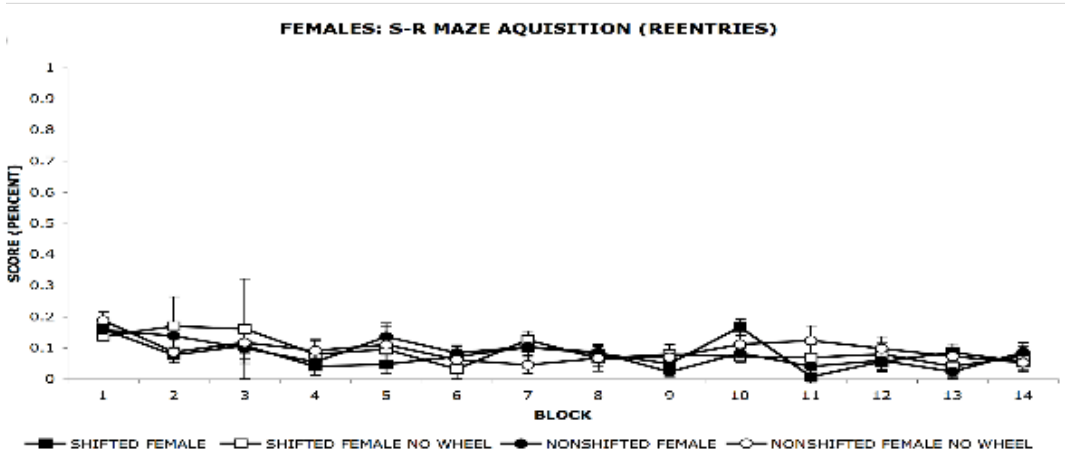

Figure 3.6b Experiment Two. The percentage of errors committed during acquisition training on the visual discrimination task developed for 8-arm radial maze for female rats only grouped by running wheel and phase-shift.

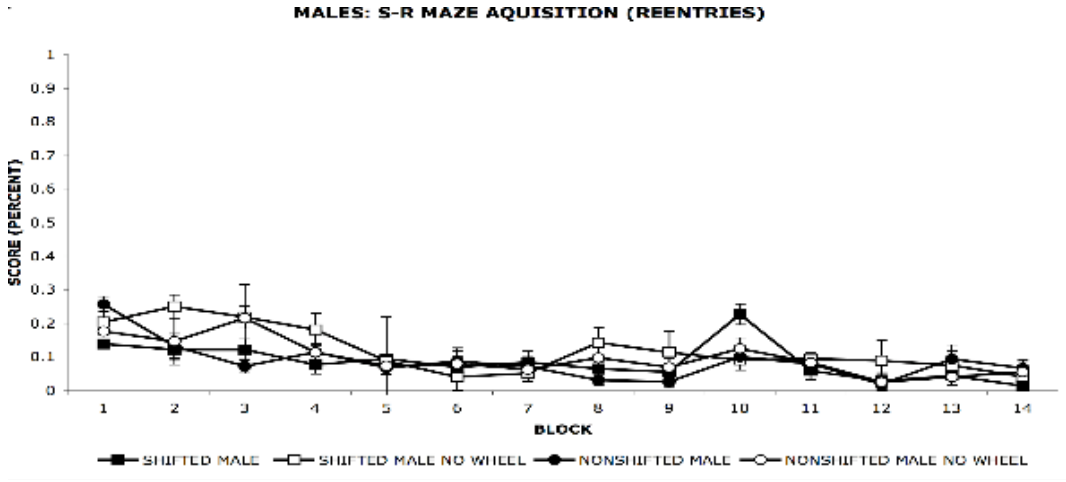

Figure 3.6c Experiment Two. The percentage of errors committed during acquisition training on the visual discrimination task developed for 8-arm radial maze for male rats only grouped by running wheel and phase-shift.

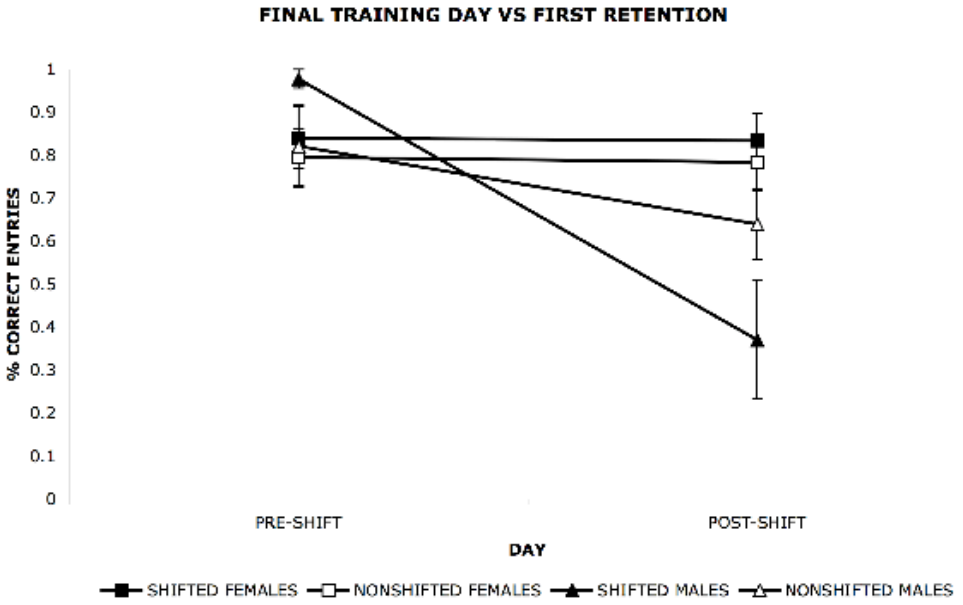

Figure 3.7a Experiment Two. Comparison for overall performance between the final day of training to the first day of retention testing following phase-shifting grouped by sex and phase-shift.

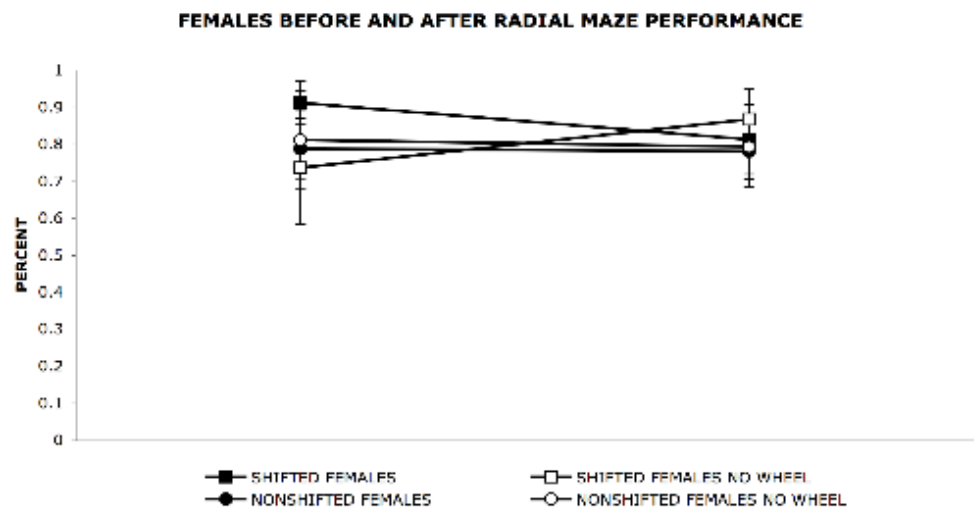

Figure 3.7b Experiment Two. Comparison for overall performance between the final day of training to the first day of retention testing following phase-shifting for female rats only grouped by phase-shift and running wheel.

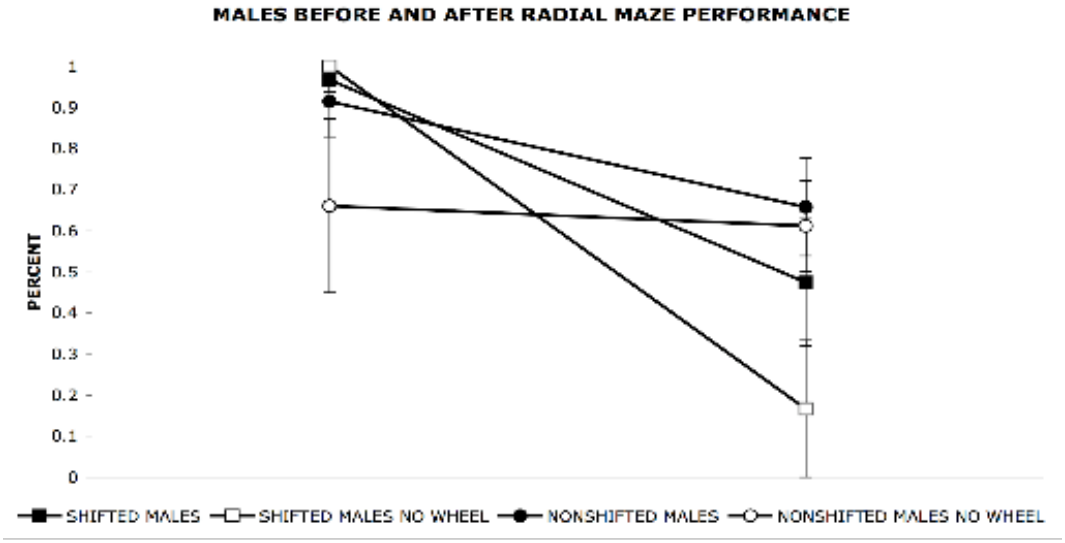

Figure 3.7c Experiment Two. Comparison for overall performance between the final day of training to the first day of retention testing following phase-shifting for male rats only grouped by phase-shift and running wheel.

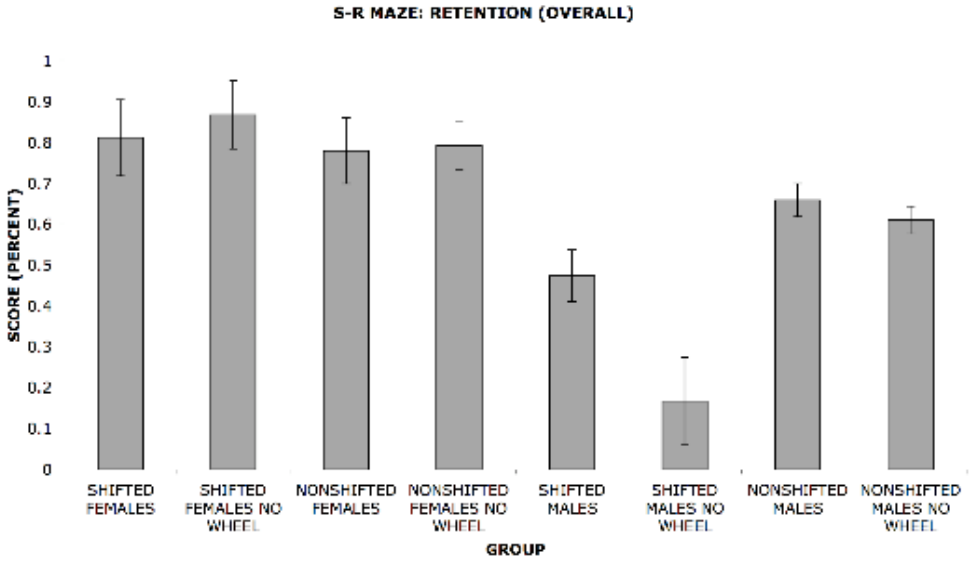

Figure 3.7d Experiment Two. Overall performance on the first day of retention testing on the visual discrimination task using the 8-arm radial maze grouped by sex, phase-shift, and running wheel.

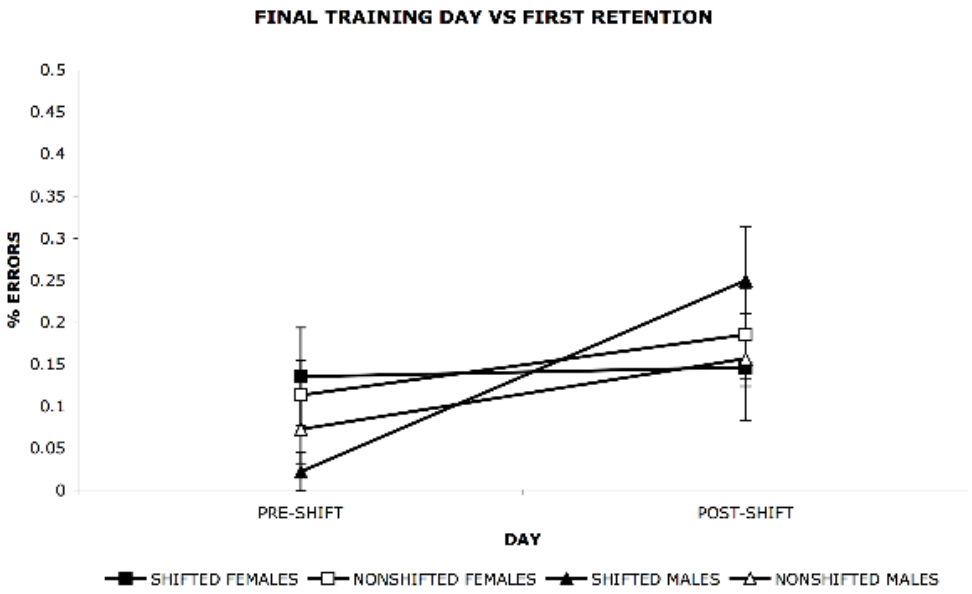

Figure 3.8a Experiment Two. Comparison of the percentage of errors committed between the final day of training to the first day of retention testing following phase-shifting, grouped by sex and phase-shift.

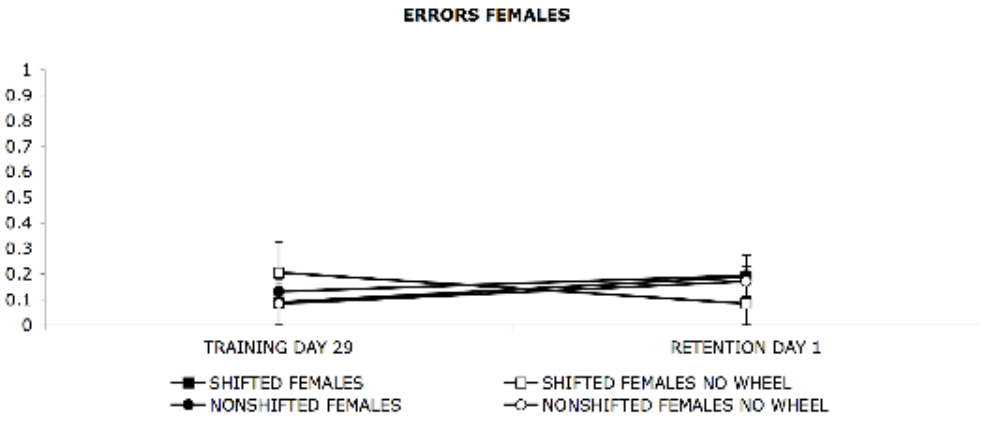

Figure 3.8b Experiment Two. Comparison of the percentage of errors committed between the final day of training to the first day of retention testing following phase-shifting for female rats only grouped by phase-shift and running wheel.

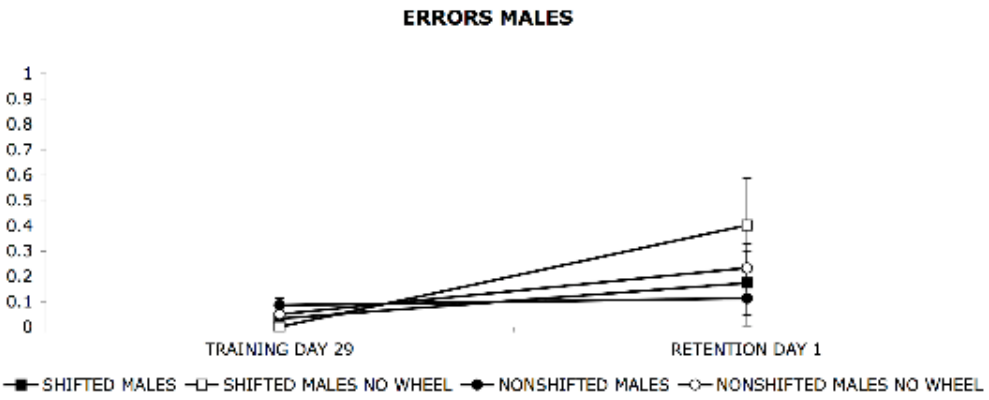

Figure 3.8c Experiment Two. Comparison of the percentage of errors committed between the final day of training to the first day of retention testing following phase-shifting for male rats only grouped by phase-shift and running wheel.

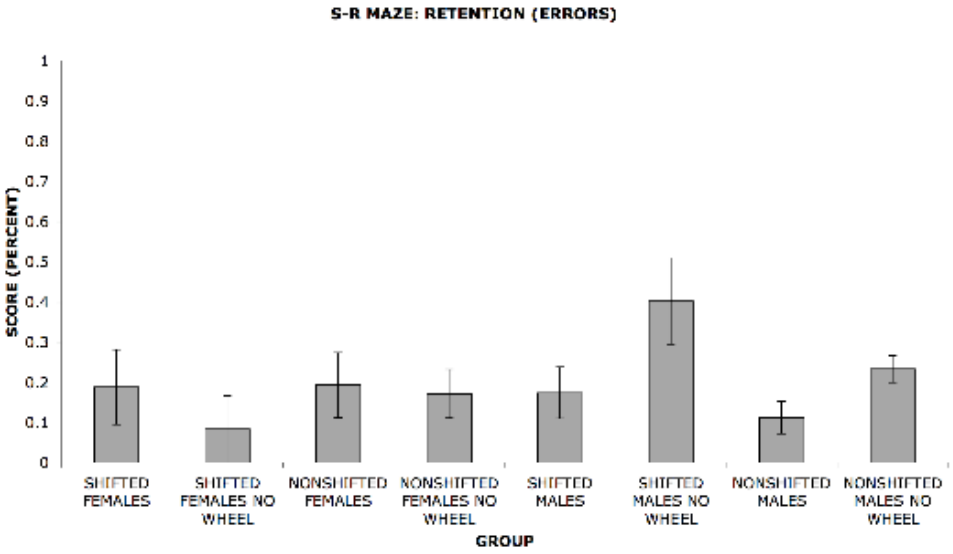

Figure 3.8d Experiment Two. The percentage of errors committed on the first day of retention testing on the visual discrimination task using the 8-arm radial maze grouped by sex, phase-shift, and running wheel.

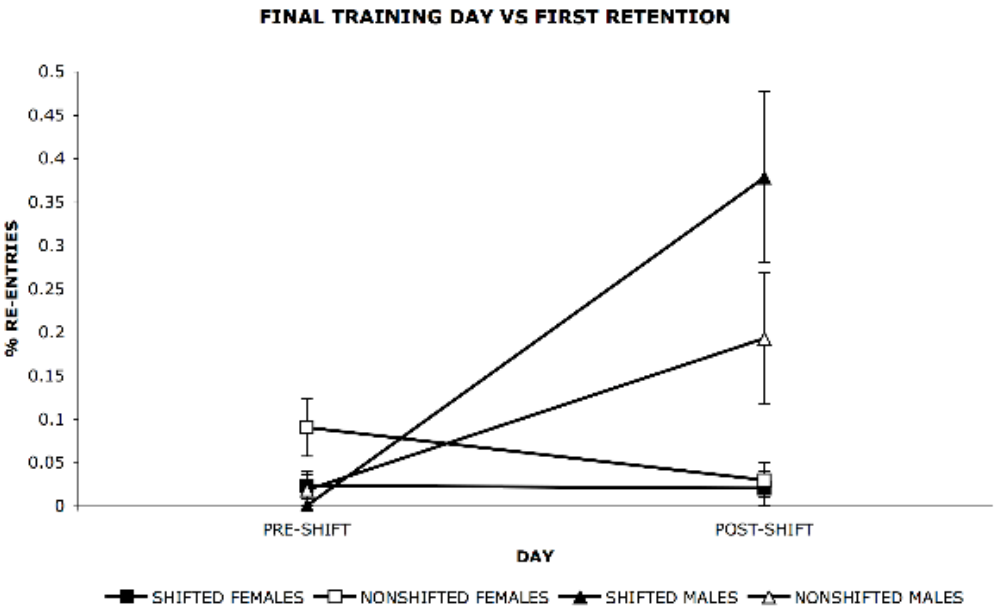

Figure 3.9a Experiment Two. Comparison of the percentage of re-entries committed between the final day of training to the first day of retention testing following phase-shifting, grouped by sex and phase-shift.

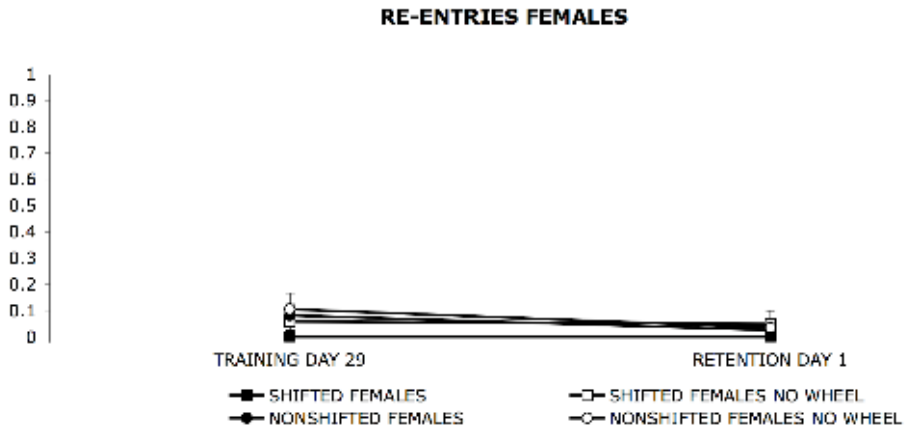

Figure 3.9b Experiment Two. Comparison of the percentage of re-entries committed between the final day of training to the first day of retention testing following phase-shifting for female rats only grouped by phase-shift and running wheel.

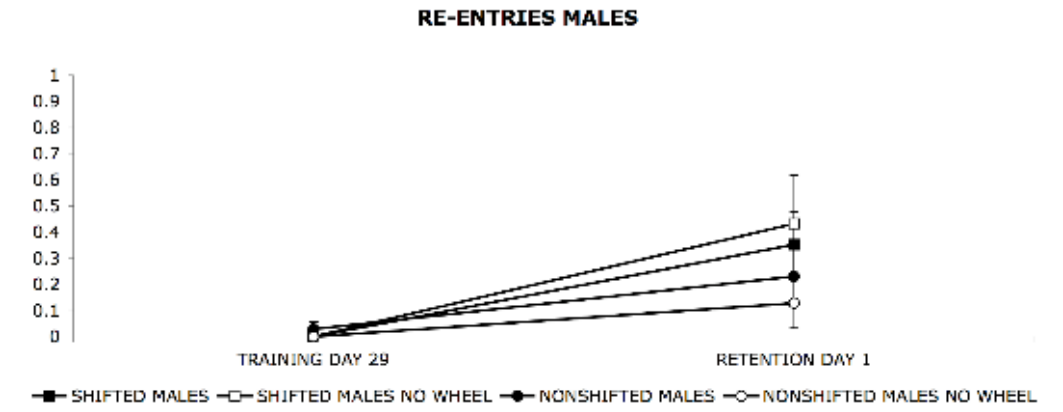

Figure 3.9c Experiment Two. Comparison of the percentage of re-entries committed between the final day of training to the first day of retention testing following phase-shifting for male rats only grouped by phase-shift and running wheel.

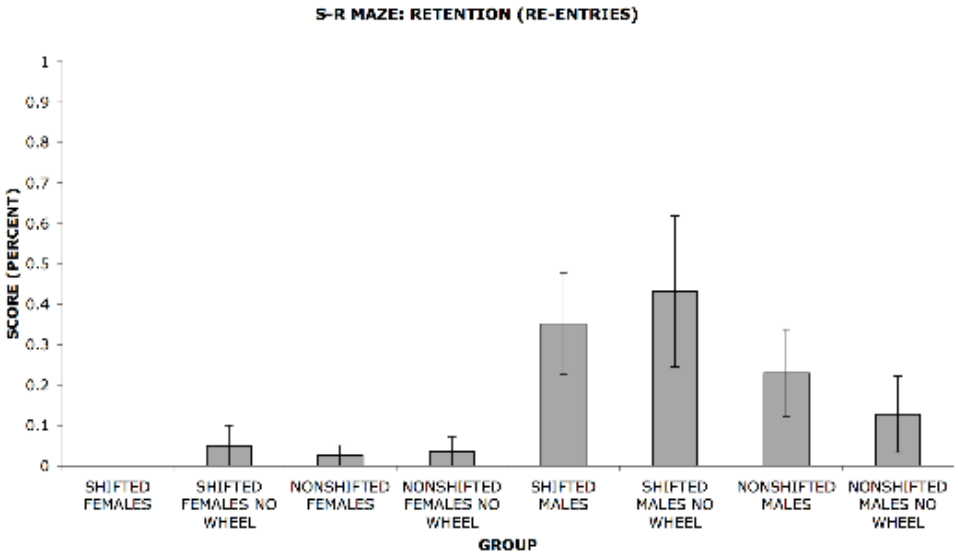

Figure 3.9d Experiment Two. The percentage of re-entries committed on the first day of retention testing on the visual discrimination task using the 8-arm radial maze grouped by sex, phase-shift, and running wheel.

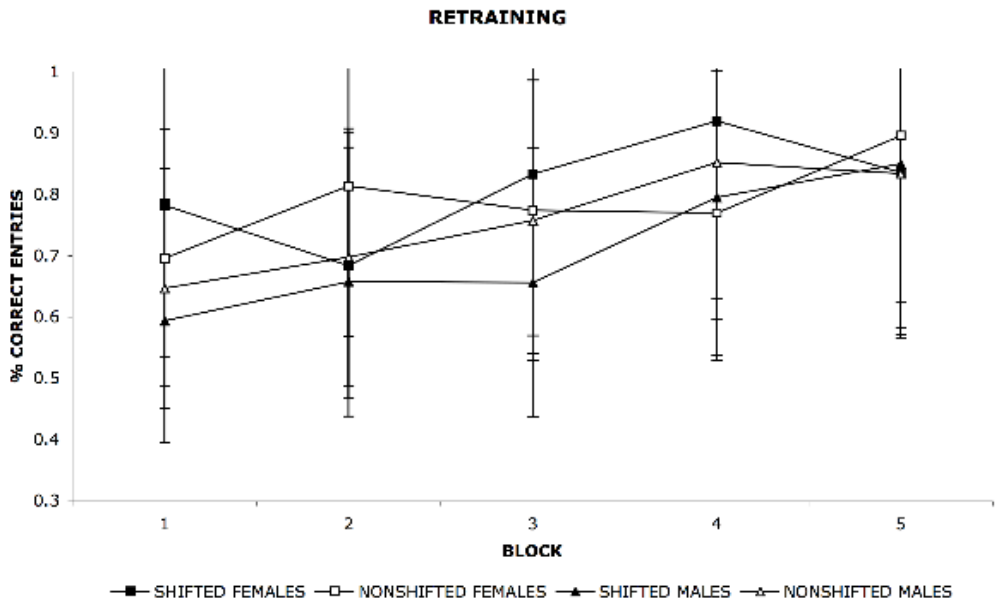

Figure 3.10a Experiment Two. The reestablishment of asymptotic performance for overall performance following phase-shifting grouped by sex and phase-shift.

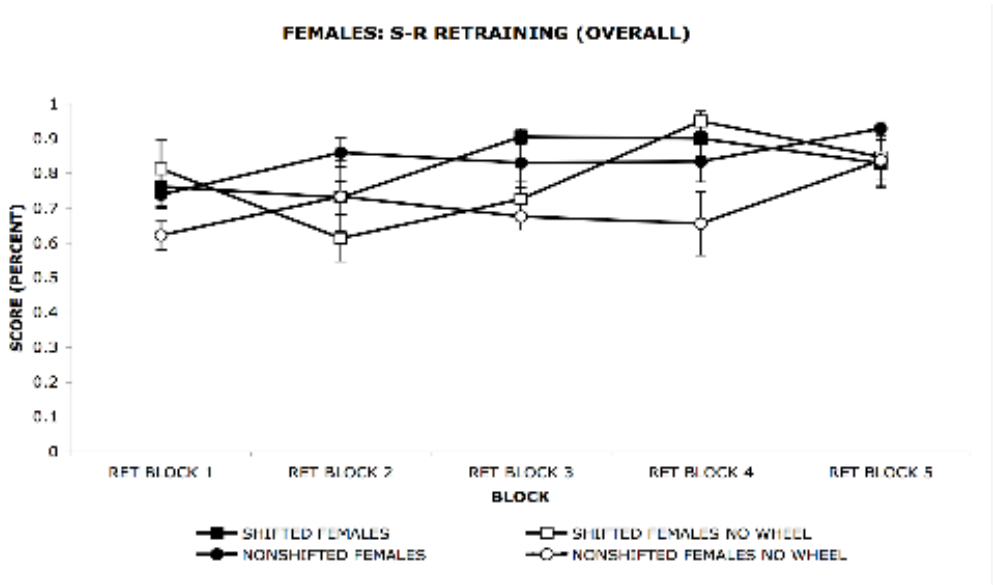

Figure 3.10b Experiment Two. The reestablishment of asymptotic performance for overall performance following phase-shifting for females only grouped by phase-shift and running wheel.

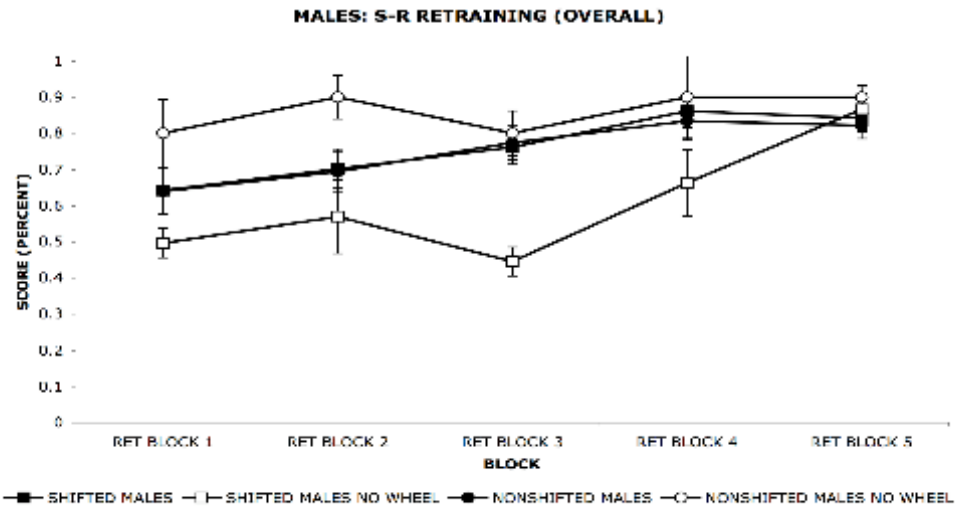

Figure 3.10c Experiment Two. The reestablishment of asymptotic performance for overall performance following phase-shifting for males only grouped by phase-shift and running wheel.

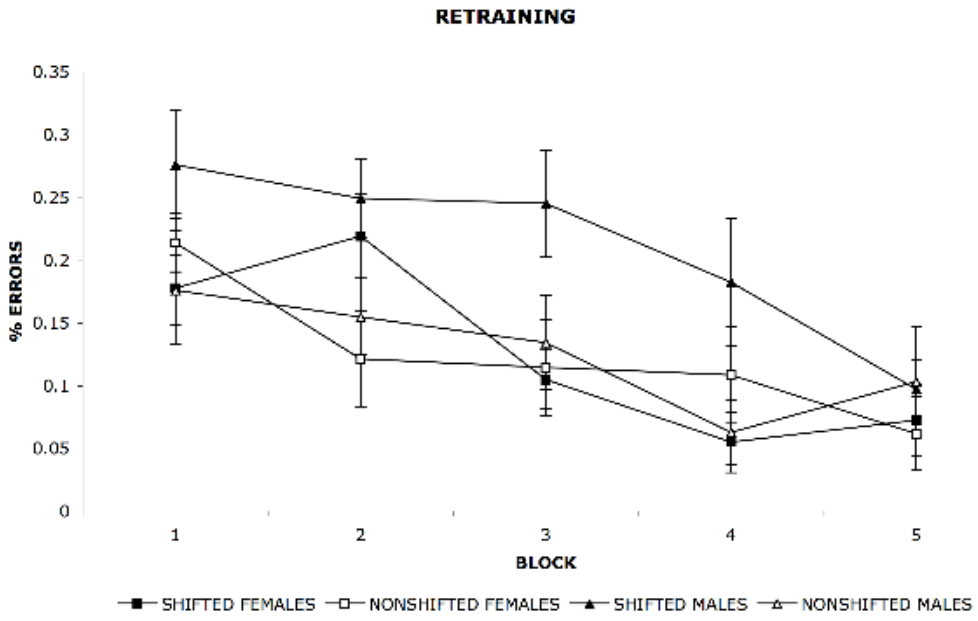

Figure 3.11a Experiment Two. The reestablishment of asymptotic performance for the percentage of errors committed following phase-shifting grouped by sex and phase-shift.

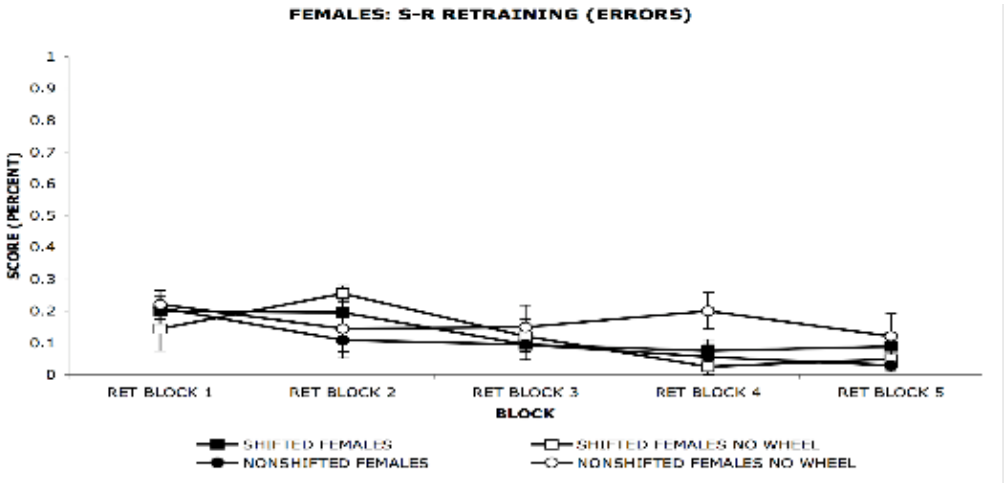

Figure 3.11b Experiment Two. The reestablishment of asymptotic performance for the percentage of errors committed following phase-shifting for females only grouped by phase-shift and running wheel.

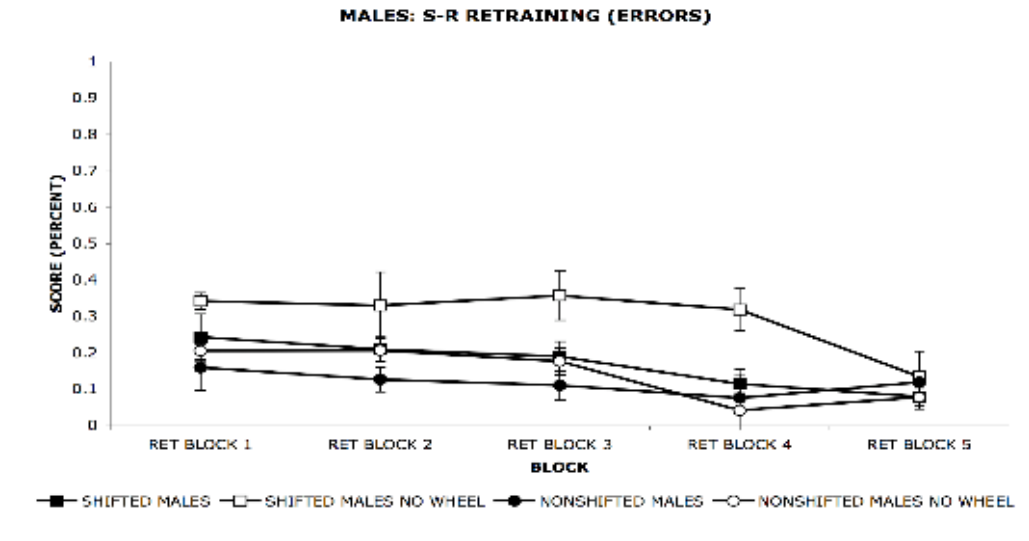

Figure 3.11c Experiment Two. The reestablishment of asymptotic performance for the percentage of errors committed following phase-shifting for males only grouped by phase-shift and running wheel.

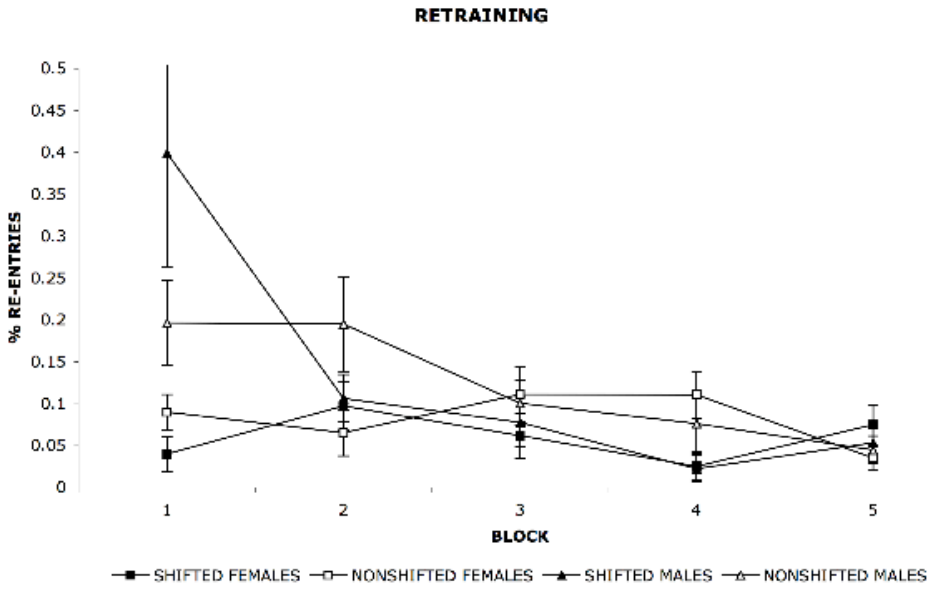

Figure 3.12a Experiment Two. The reestablishment of asymptotic performance for the percentage of re-entries committed following phase-shifting grouped by sex and phase-shift.

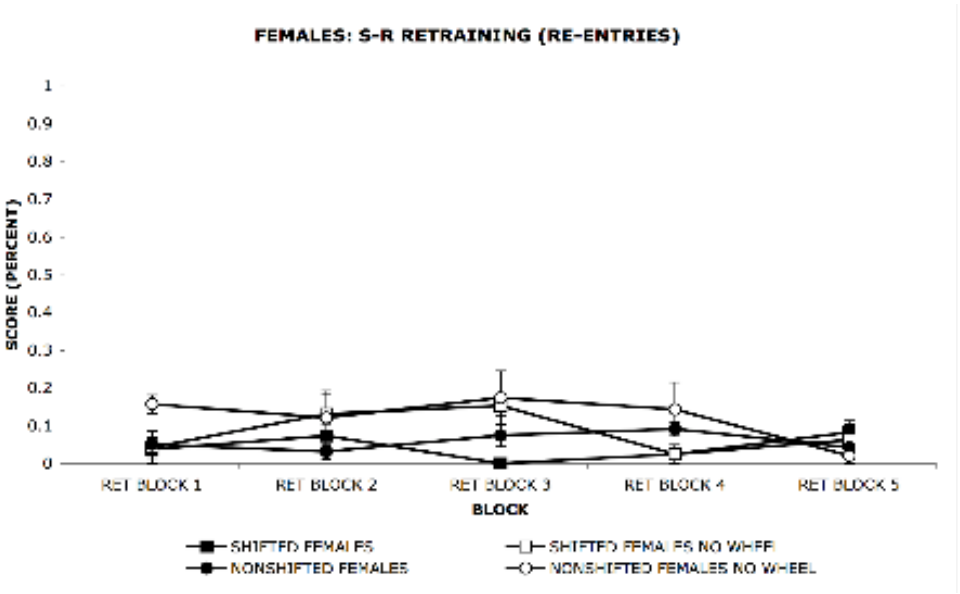

Figure 3.12b Experiment Two. The reestablishment of asymptotic performance for the percentage of re-entries committed following phase-shifting for females only grouped by phase-shift and running wheel.

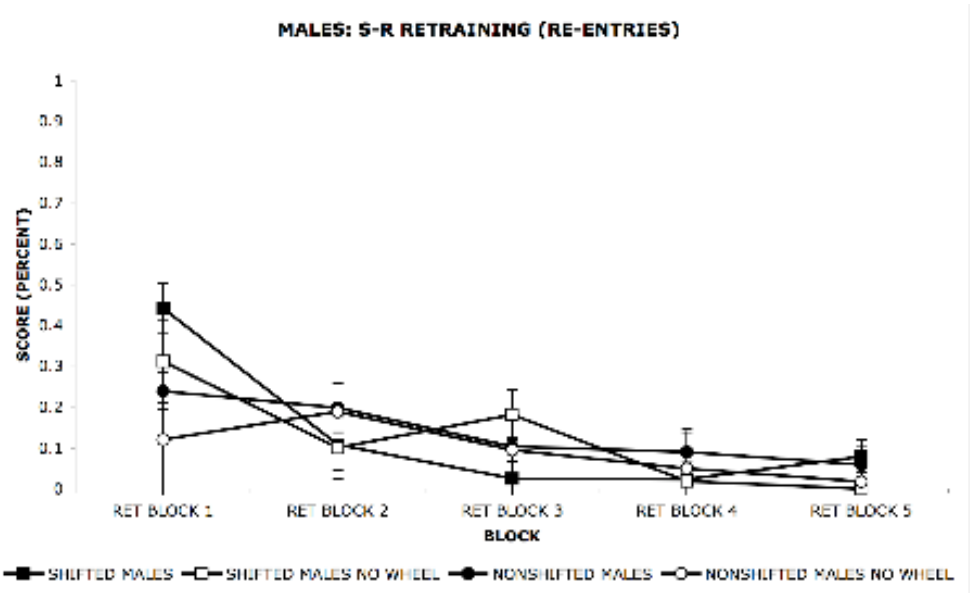

Figure 3.12c Experiment Two. The reestablishment of asymptotic performance for the percentage of re-entries committed following phase-shifting for males only grouped by phase-shift and running wheel.

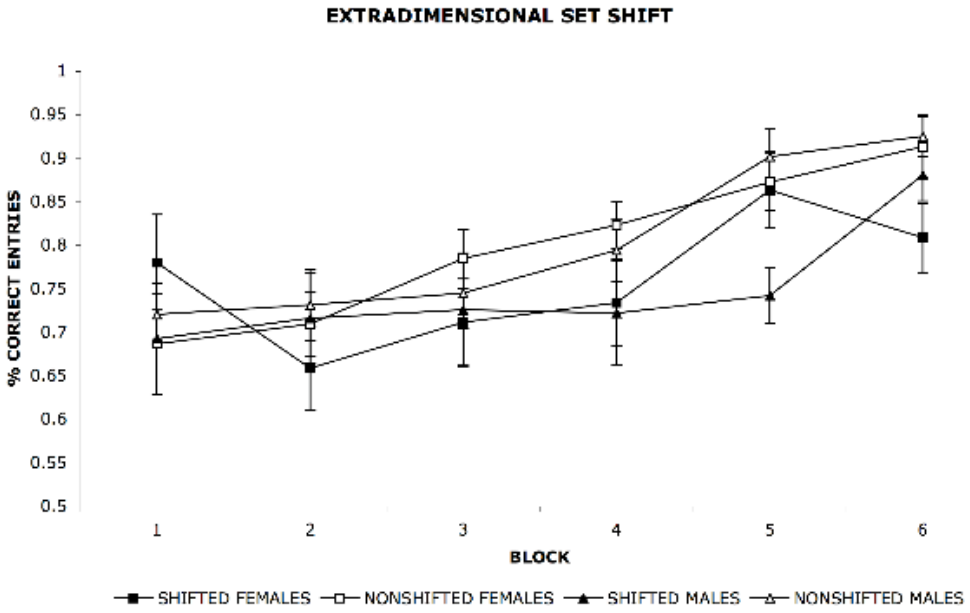

Figure 3.13a Experiment Two. Overall performance of the extradimensional attentional set shift condition grouped by sex and phase-shift.

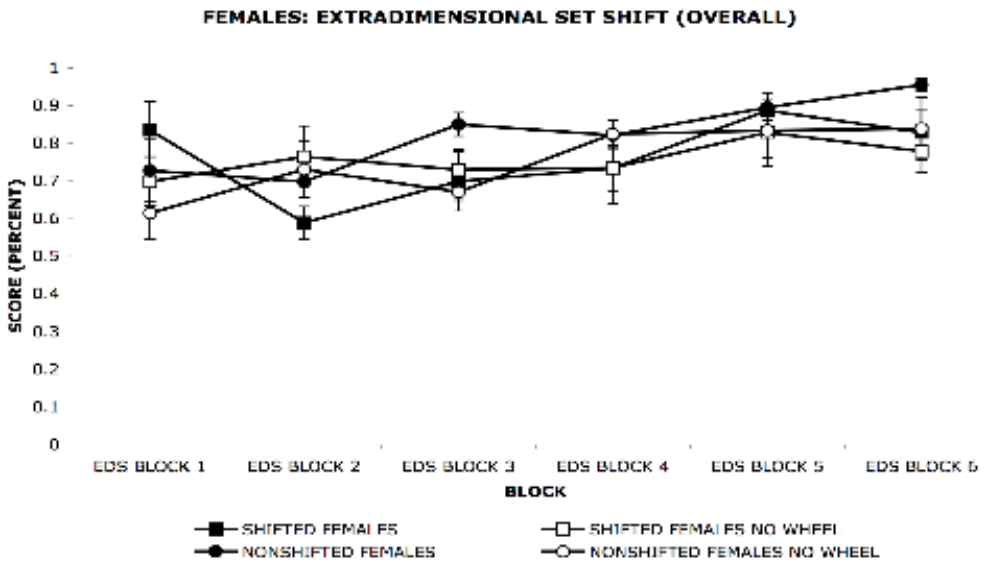

Figure 3.13b Experiment Two. Overall performance of the extradimensional attentional set shift condition for female rats only on overall performance grouped by phase-shift and running wheel.

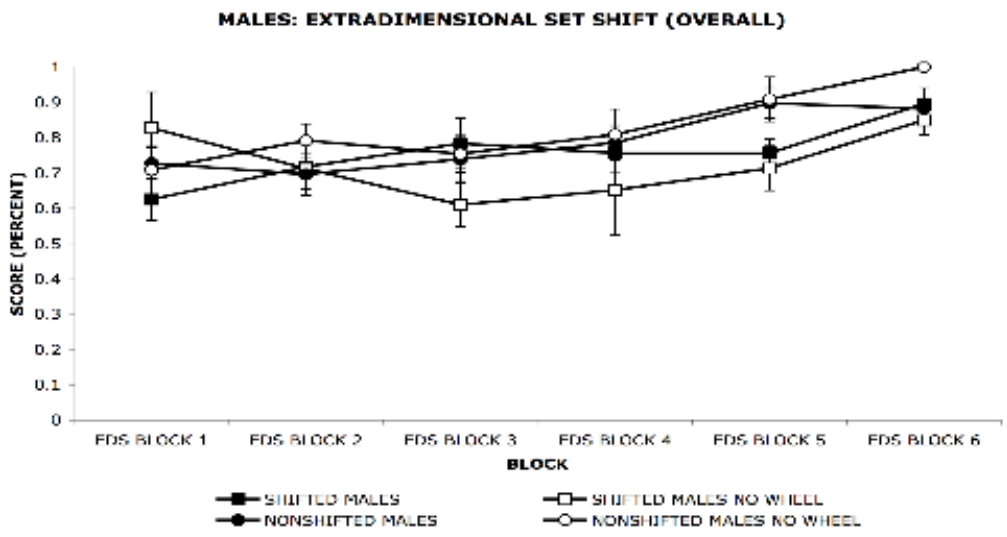

Figure 3.13c Experiment Two. Overall performance of the extradimensional attentional set shift condition for male rats only on overall performance grouped by phase-shift and running wheel.

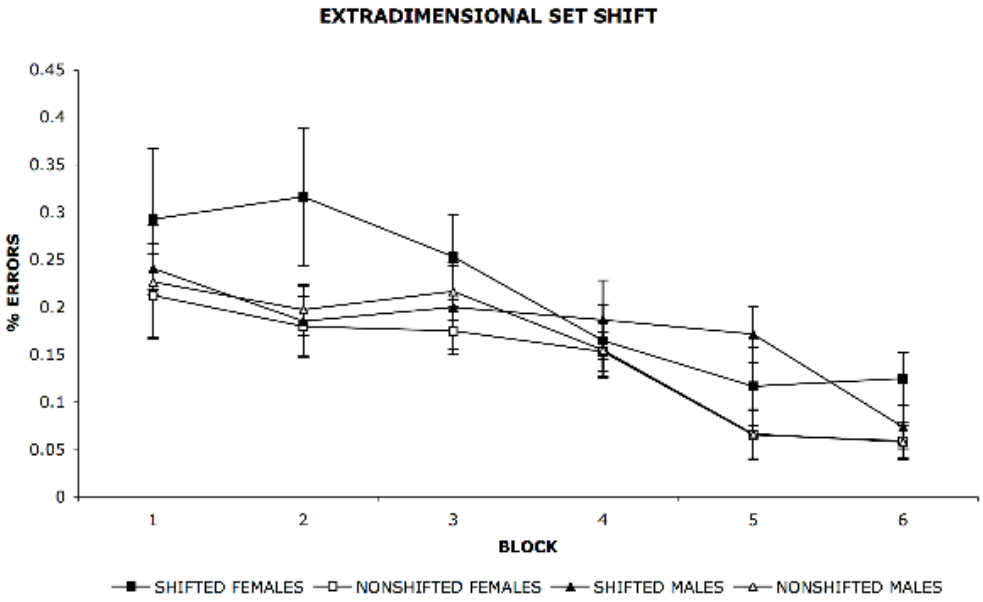

Figure 3.14a Experiment Two. The percentage of errors committed during acquisition of the extradimensional attentional set shift condition grouped by sex and phase-shift.

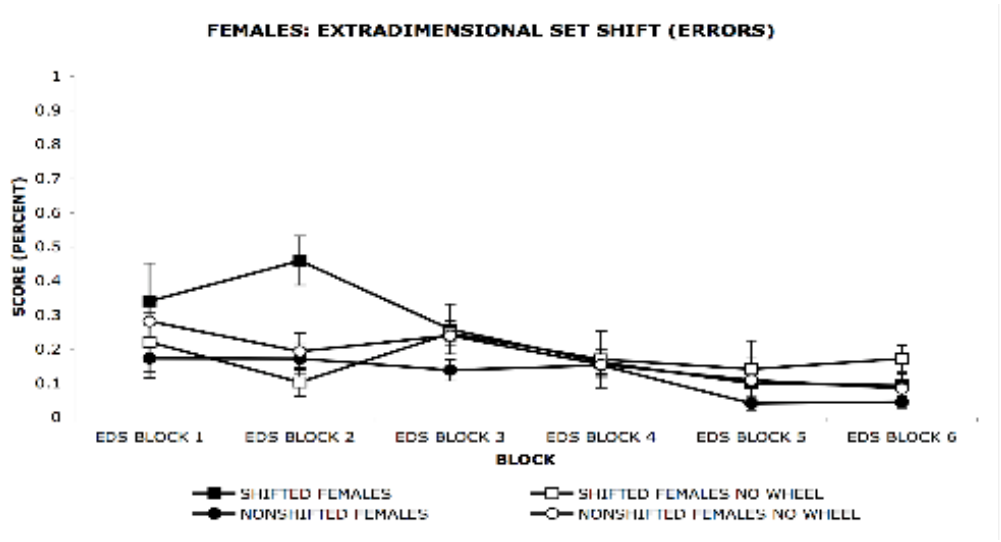

Figure 3.14b Experiment Two. The percentage of errors committed during acquisition of the extradimensional attentional set shift condition for female rats only grouped by phase-shift and running wheel.

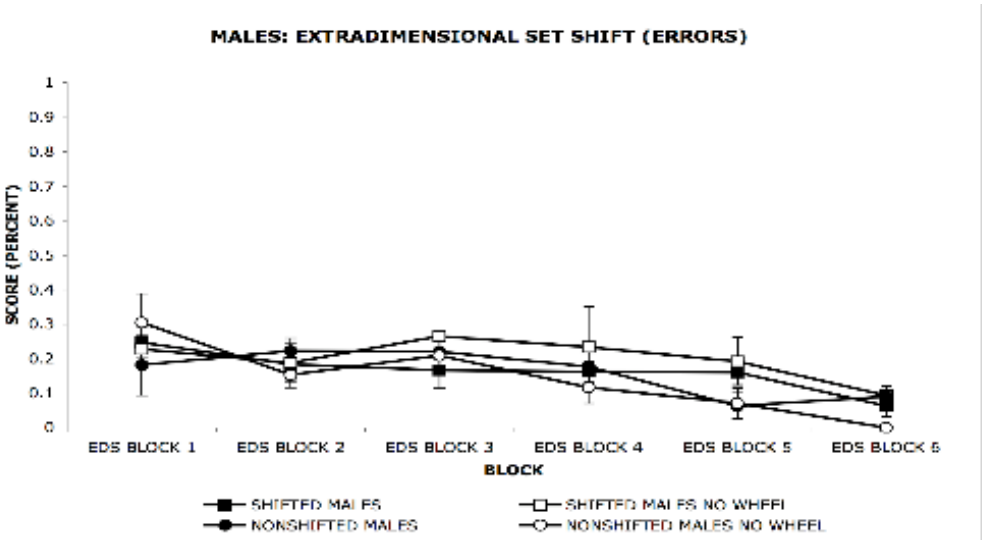

Figure 3.14c Experiment Two. The percentage of errors committed during acquisition of the extradimensional attentional set shift condition for male rats only grouped by phase-shift and running wheel.

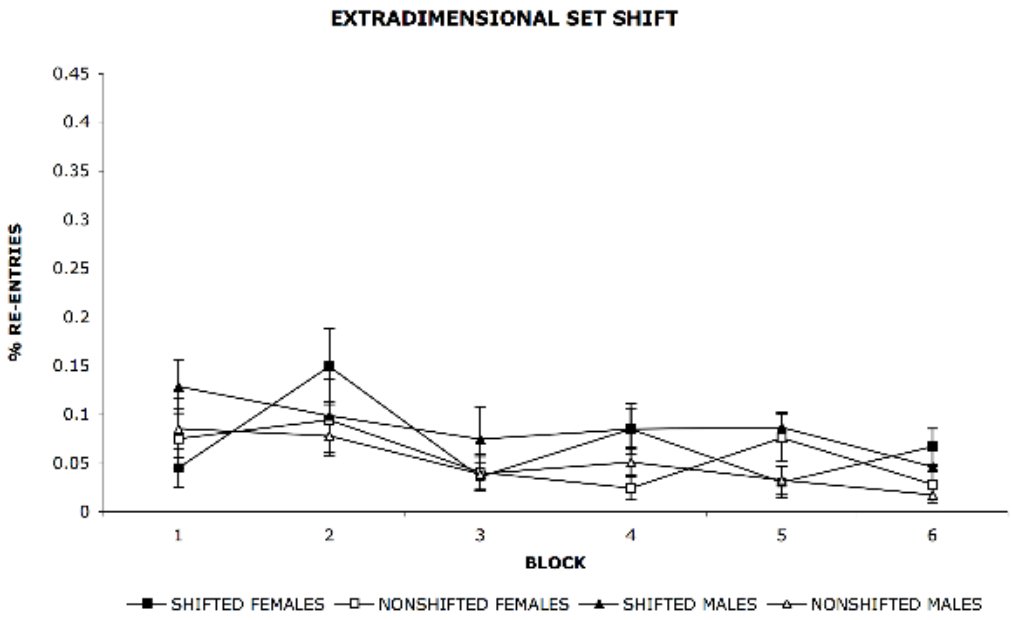

Figure 3.15a Experiment Two. The percentage of re-entries committed during acquisition of the extradimensional attentional set shift condition grouped by sex and phase-shift.

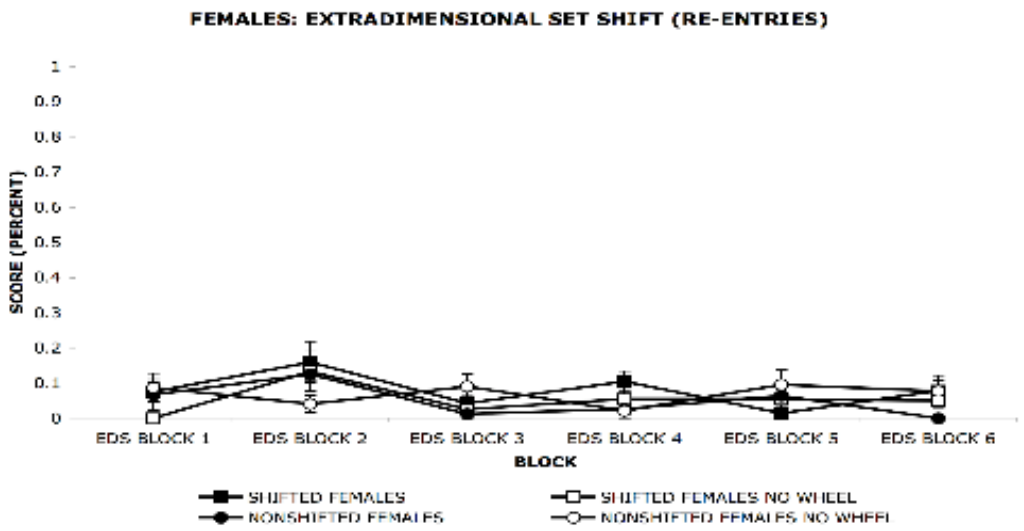

Figure 3.15b Experiment Two. The percentage of re-entries committed during acquisition of the extradimensional attentional set shift condition for female rats only grouped by phase-shift and running wheel.

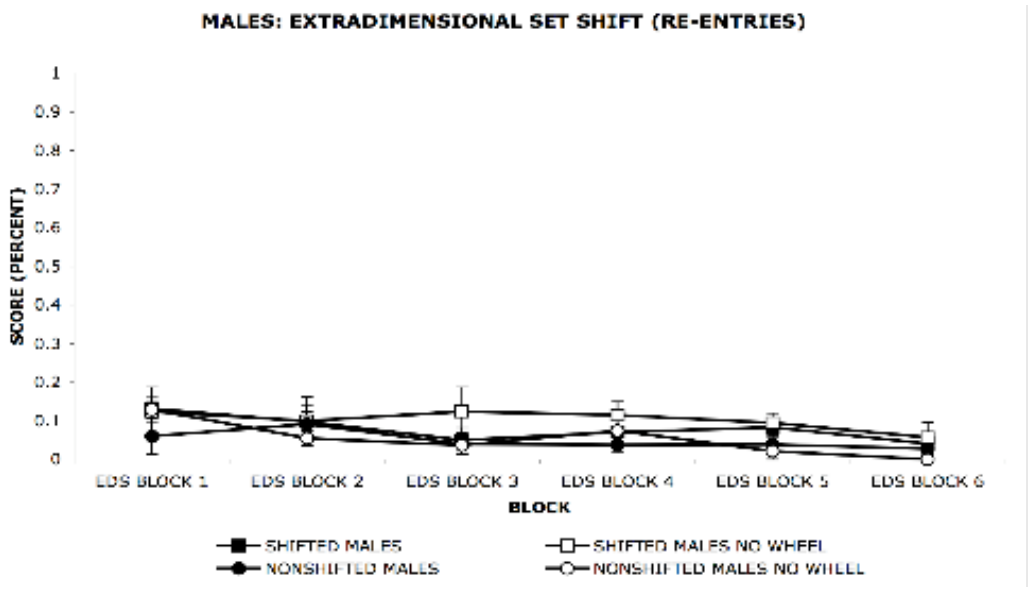

Figure 3.15c Experiment Two. The percentage of re-entries committed during acquisition of the extradimensional attentional set shift condition for male rats only grouped by phase-shift and running wheel.

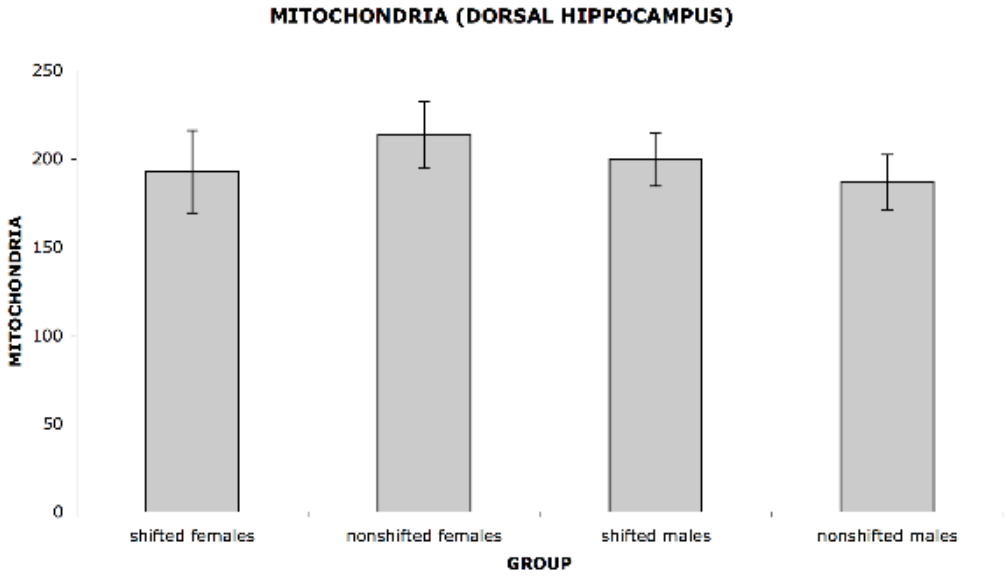

Figure 3.16a Experiment Two. Assessment of mitochondrial density in dorsal hippocampus.

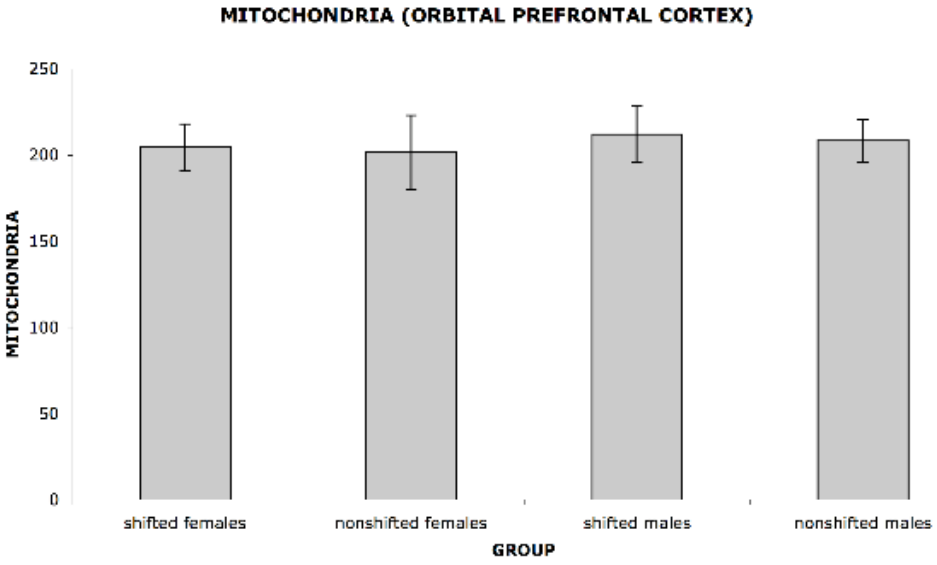

Figure 3.16b Experiment Two. Assessment of mitochondrial density in orbital prefrontal cortex

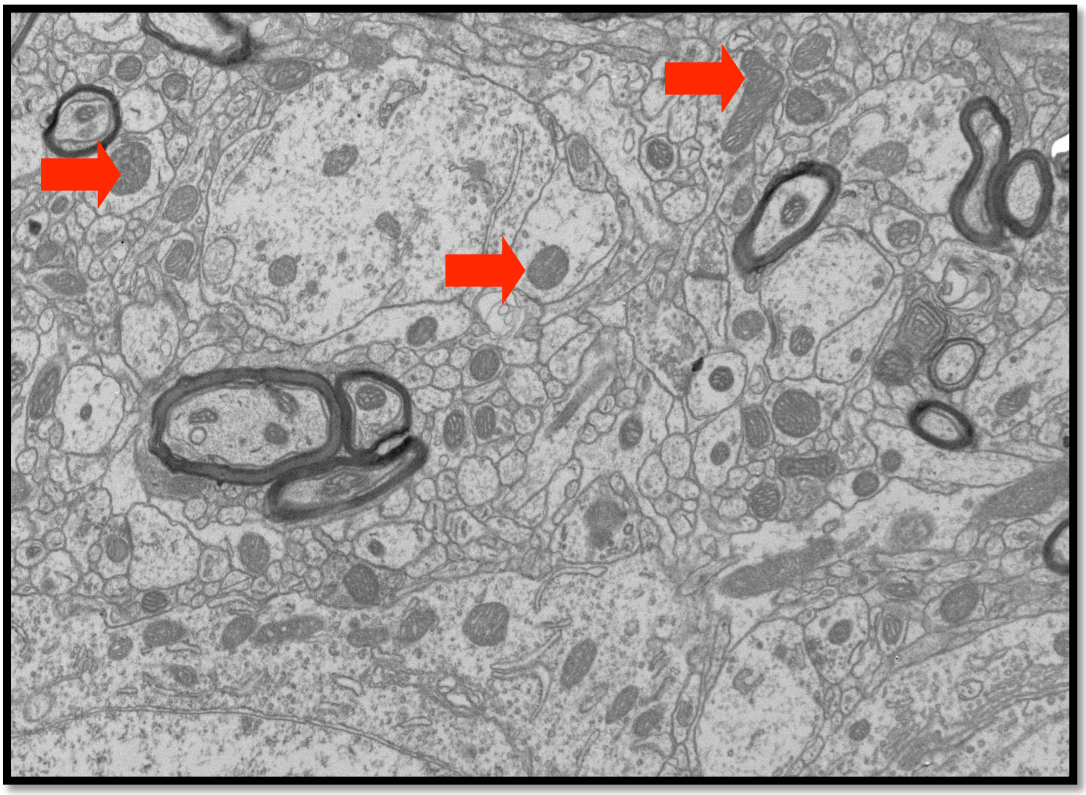

Figure 3.17 Experiment Two. Photomicrograph depicting mitochondrial density in orbital prefrontal cortex. The red arrows indicate the location of three mitochondria. No significant differences were observed in association with sex, phase-shift, or running wheel.

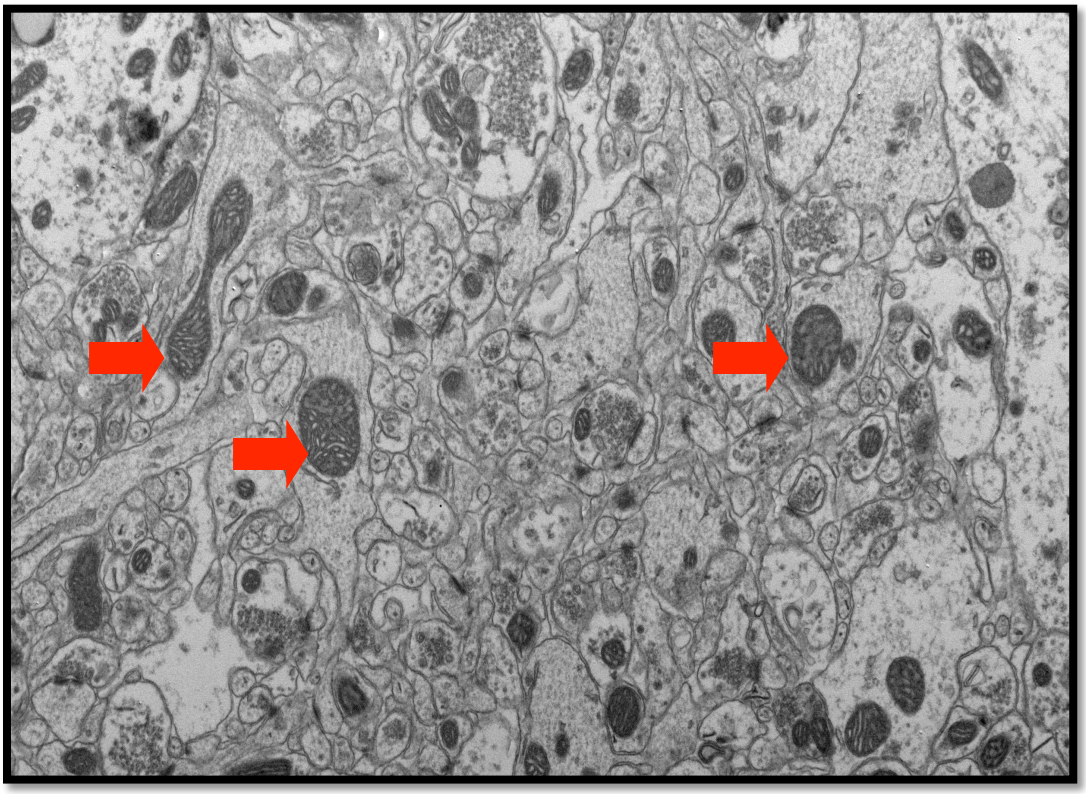

Figure 3.18 Experiment Two. Photomicrograph depicting mitochondrial density in dorsal hippocampus. The red arrows indicate the location of three mitochondria. No significant differences were observed in association with sex, phase-shift, or running wheel.

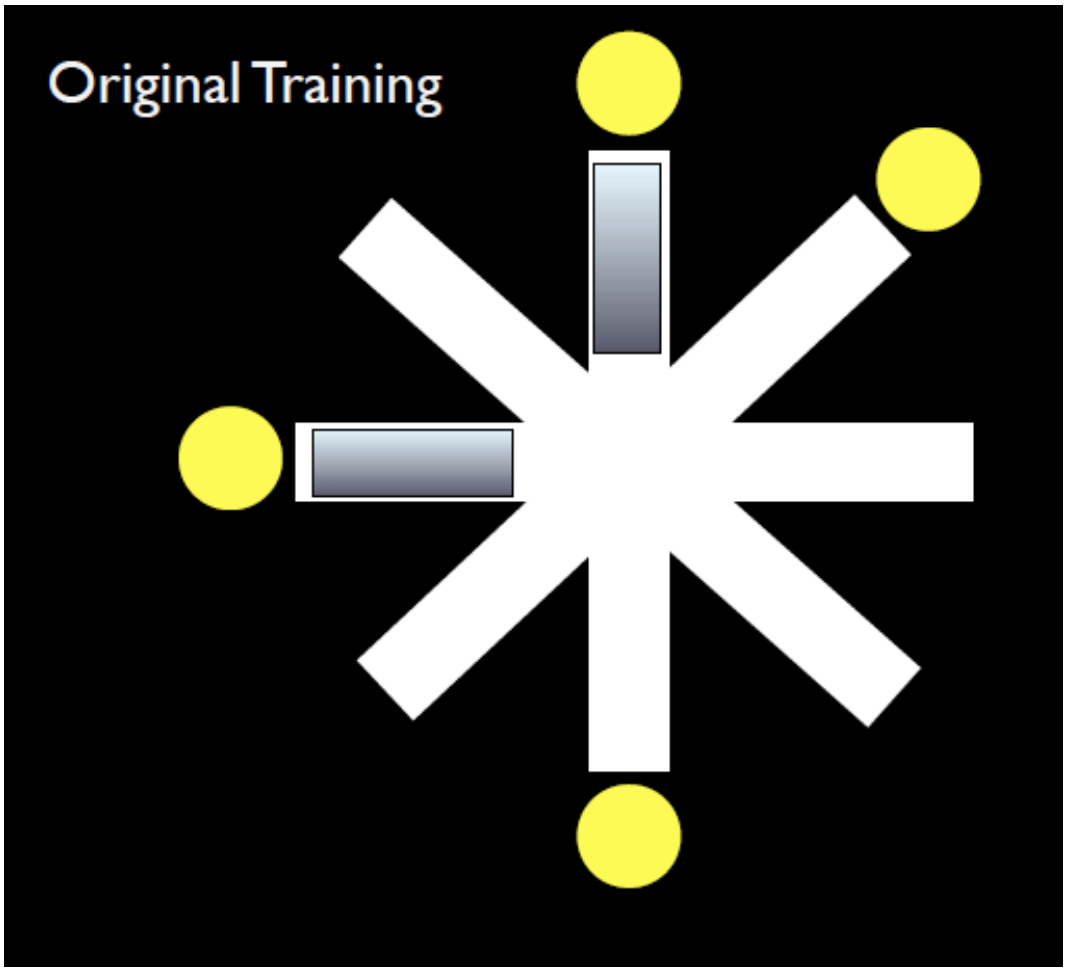

Figure 4.1a Appendix. Figure depicting the experimental conditions during original acquisition of the visual discrimination task using the 8-arm radial maze.

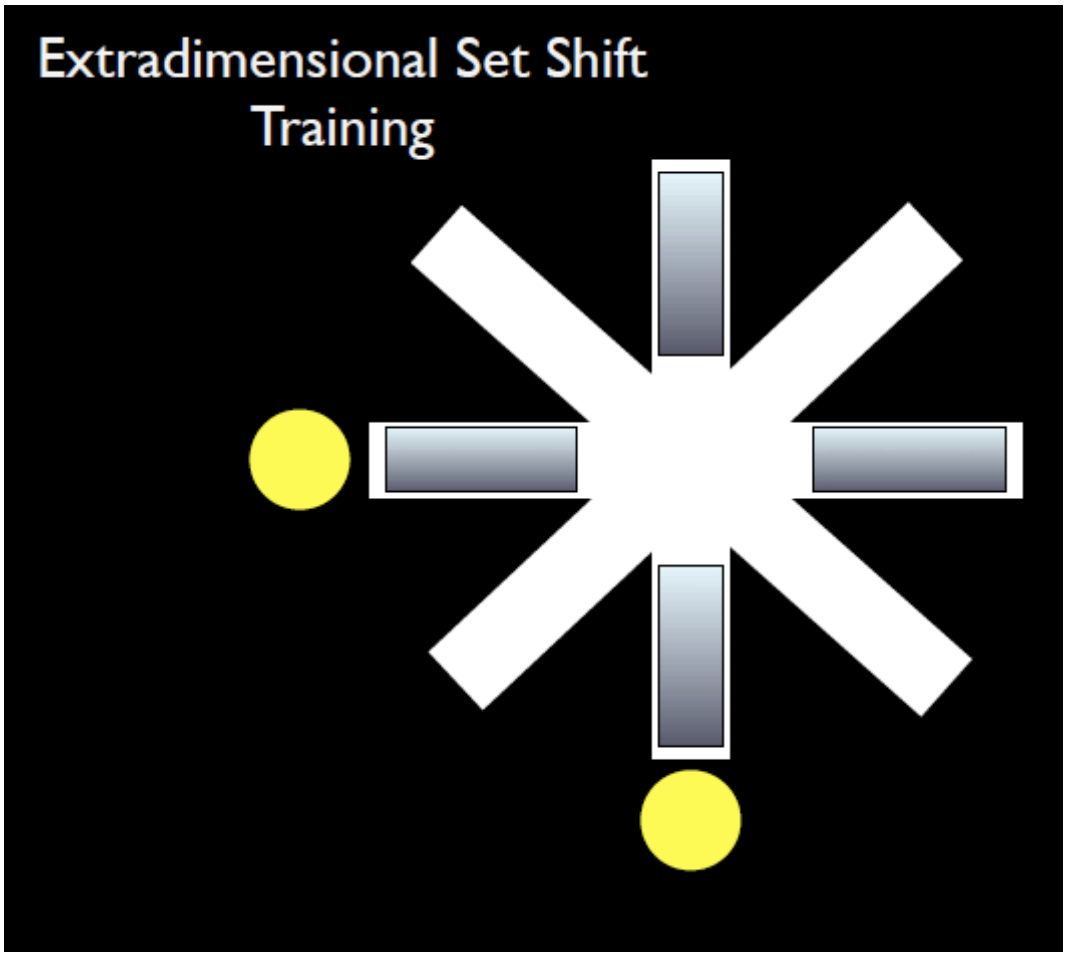

Figure 4.1b Appendix. Figure depicting the experimental conditions during the extradimensional attentional set shift task using the 8-arm radial maze.

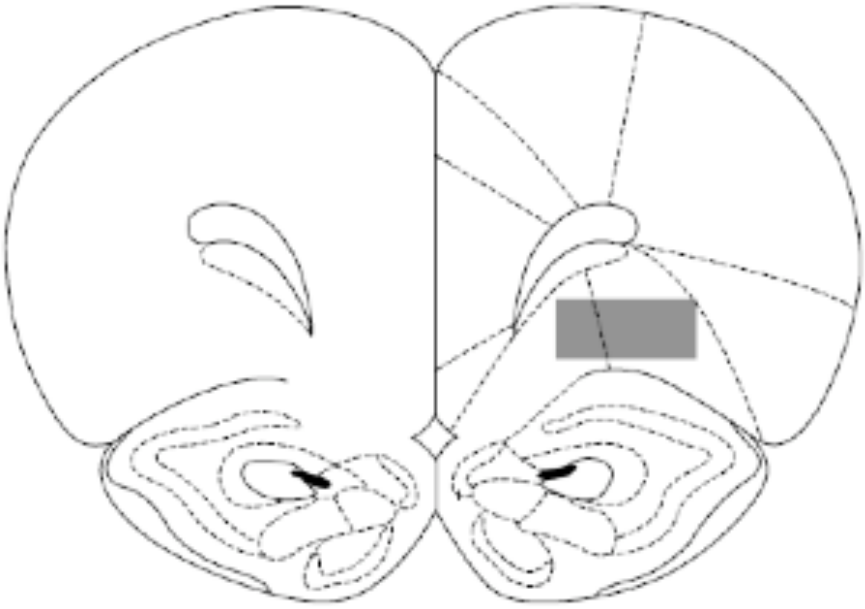

Figure 4.2a The OPFC region selected for electron microscopy.

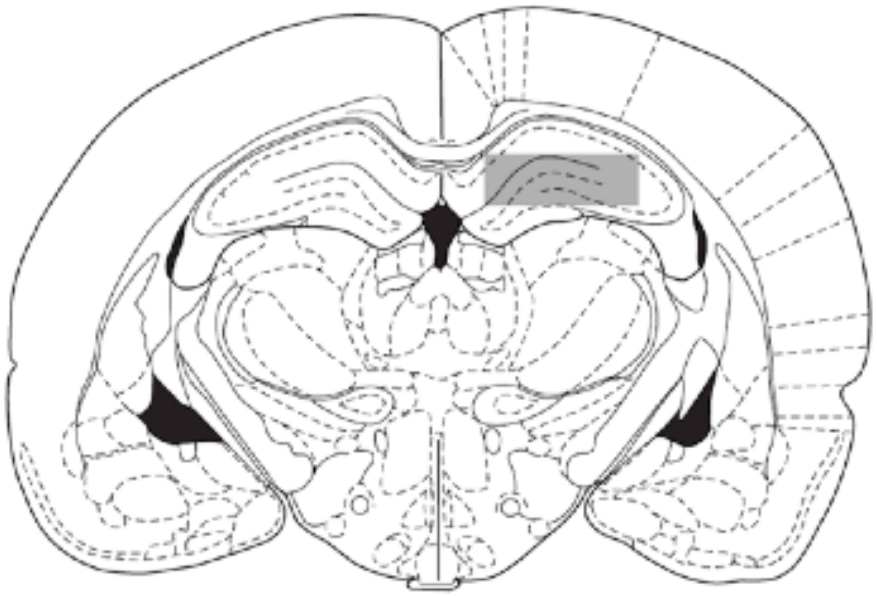

Figure 4.2b The region of HPC selected for electron microscopy.