CAN THE FOOD-ENTRAINABLE OSCILLATOR AMELIORATE THE DELETERIOUS EFFECT OF CIRCADIAN RHYTHM DISRUPTION IN AN ANIMAL MODEL OF SOCIAL JET LAG?

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Abstract

Today's society fosters circadian rhythm disruptions. In particular, individuals often experience "social jet lag" (SJL), resulting in differences between weekday and weekend sleep schedules, and disruption of their light-entrainable oscillator (LEO). The current study investigated the interaction between the LEO and the food-entrainable oscillator (FEO) by implementing a novel animal model of SJL, the "social jet lag manipulation" (SJM). Particularly, the impact of SJL on retention and acquisition of hippocampal-dependent and non-hippocampal-dependent tasks was investigated. During Experiment One, while receiving one (FEO access) or many (no FEO access) meals per day, rats were trained under a 12:12 light-dark cycle, then exposed to the SJM. SJM and Control rats retained the tasks equally, yet rats with FEO access retained hippocampal-dependent tasks better than rats without FEO access. During Experiment Two, while receiving one or many meals per day, rats were exposed to the SJM during hippocampal-dependent and non-hippocampal-dependent task training. SJM and Control rats acquired the tasks equally. However, rats with FEO access acquired the hippocampal-dependent task faster than rats without FEO access. While no detrimental impact of the SJM was apparent, actograms suggested that the SJM induced free-running activity. Previous shift work models have induced free-running activity, causing hippocampal-dependent learning and memory deficits. It is, therefore, possible that the hippocampal-dependent tasks used in the current study were not sensitive enough to display hippocampal deficits. Nonetheless, the current findings displaying the benefit of FEO access for learning and memory indicate the need to further investigate this oscillator and its potential benefits.

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While the discovery of electricity and subsequent invention of light bulbs has advanced society in innumerable ways, it has also developed an environment that fosters sleep and circadian rhythm irregularity. This disruption in circadian rhythms has become even more pronounced since the use of technology in homes has become commonplace (Hirotsu, Tufik, & Anderson, 2015). Furthermore, the increased demands placed on individuals by their socioeconomic requirements and societal pressures further exacerbate the disruption of circadian rhythms (Spiegel, Knutson, Leproult, Tasali, & Van Cauter, 2005). As circadian rhythm disruption has been associated with a wide variety of health complications, and as increased socioeconomic demands and technological advancements are unlikely to dissipate, it is imperative that we attempt to further understand the effect that circadian rhythm disruption has on health and cognitive functioning (Potter et al., 2016).

The advantages of a functional circadian system are evidenced by its conservation throughout evolution, as the earliest life forms displayed environmental adaption characteristic of circadian rhythmicity (Mohawk, Green, & Takahashi, 2012). As such, the organization of the circadian system is very intricate, ranging from single genes to overt behaviour (Mohawk et al., 2012). In fact, a circadian clock is located within practically every cell of the human body (Welsh, Yoo, Liu, Takahashi, & Kay, 2004). Within such cells, endogenous circadian rhythms are produced by an autoregulatory transcription/post-transcription/translation/post-translation-based feedback cycle involving *Clock, Bmal1, Period* (*Per1* and *Per2*) and *Cryptochrome* (*Cry1* and *Cry2*)

genes (Okamura, 2004; Mohawk et al., 2012). This feedback cycle takes approximately 24 hours to complete, hence the notion that circadian rhythms have a cycle of 24 hours (Dibner, Schibler, & Albrecht, 2010).

Such 24-hour cycles provide temporal organization for a variety of regulatory biological processes. To ensure proper regulation of these biological processes, the circadian system is hierarchically organized. The circadian clock, or oscillator, at the top of the hierarchy, also known as the "master clock", is the suprachiasmatic nucleus (SCN) (Mulder, ReckMan, Gerkema, & Van der Zee, 2015). The SCN is responsible for controlling behavioural rhythms and synchronizing subordinate circadian oscillators (Mohawk et al., 2012; Mulder et al., 2015). The SCN is located within the periventricular zone of the anterior hypothalamus and receives afferent projections from the geniculohypothalamic tract and median raphe nucleus (Zelinski, Deibel, & McDonald, 2014). The SCN projects to the paraventricular hypothalamic nucleus, dorsomedial hypothalamic nucleus, medial preoptic area, anteroventral periventricular nucleus, lateral septal nucleus, ventral lateral geniculate nucleus and ventral tegmental area via the medial preoptic nucleus (Luo & Aston-Jones, 2009; Watts, Swanson, & Sanchez-Watts, 1987).

The SCN entrains, or synchronizes, to the external environment through information regarding the environmental light/dark (LD) cycle. Such information is received by photoreceptors in the eye and passed along the retino-hypothalamic tract to the SCN (Angeles-Castellanos, Salgado-Delgado, Rodriguez, Buijs, & Escobar, 2010; Davidson, Castanon-Cervantes, Feise, Molyneus, & Harrington, 2009; Mulder, Papantoniou, Gerkema, & Van der Zee, 2014). The SCN is so influential that when it is

lesioned, both physiological and behavioural circadian rhythmicity are lost entirely (Moore & Eichler, 1972; Stephan & Zucker, 1972). However, when SCN tissue is transplanted in an animal with an SCN lesion, rhythmicity returns (Ralph, Foster, Davis, & Menaker, 1990). As the primary zeitgeber (exogenous cue used for entrainment) of the SCN is the LD cycle, the SCN is often referred to as the Light-Entrainable Oscillator (LEO).

While the LEO entrains to the light environment, subordinate circadian oscillators entrain to different, non-photic zeitgebers. Some of these non-photic zeitgebers include food, exercise, and caffeine consumption (Aschoff et al., 1971). It has been well established in animal research that a Food-Entrainable Oscillator (FEO) exists (Mistlberger, de Groot, Bossert, & Marchant, 1996). Access to the FEO is established in rats by providing two or fewer meals per day, at the same time each day (Bolles & Moot, 1973). Entrainment to mealtimes by the FEO results in rhythmic increases in general activity prior to expected meal times, which is known as food-anticipatory activity (FAA) (Bolles & Moot, 1973; Silver & Kriegsfeld, 2014). FAA persists even after SCN ablation, indicating that the FEO and LEO operate through different brain loci, however the exact anatomical locus of the FEO is still unknown (Mistlberger et al., 1996; Stephen, 2002). Moreover, access to the FEO in rats is known to improve learning and memory on a time-place learning (TPL) task (Wall, Lewis, Deibel, Hallett, & Thorpe, 2018). Furthermore, when meals were delayed in human participants, *Per2* mRNA rhythms in adipose tissue were subsequently delayed, indicating that regulated meal timing is involved in the synchronizing of human peripheral circadian rhythms (Wehrens et al., 2017). This is, therefore, indicative of the presence of an FEO in humans.

Central and peripheral circadian clocks play an integral role in a plethora of regulatory biological processes throughout the body. Specifically, biological processes such as the sleep-wake cycle, hormone regulation, gene expression, immune functioning and body temperature are all controlled by the circadian system (Sharma, Tiwari, & Singaravel, 2015; Waterhouse et al., 2001; Wittmann, Dinich, Merrow, & Roenneberg, 2006; Young & Kay, 2001).

As circadian rhythms play a major role in fundamental regulatory biological processes, disruption of circadian rhythms can be detrimental to overall health (Potter et al., 2016; Roenneberg & Merrow, 2016). In fact, both the severity and timing of cardiovascular infarcts vary based on circadian time, further displaying the intricate relationship between cardiovascular health and circadian rhythms (Zelinski et al., 2014). Furthermore, sudden changes in LD cycles, or chronic transmeridian flights, have been associated with increased levels of cortisol (Caufriez et al., 2002; Cho, 2001; Nestler et al., 2002). Likewise, a variety of cancers, including colorectal, prostate, pancreatic, breast and skin cancers, have been both epidemiologically and experimentally associated with circadian disruption (Davis & Mirick, 2006; Wood et al., 2010; Zelinski et al., 2014). More specifically, circadian disruption has been associated with decreased survival rates in cancer patients (Mormont & Levi, 2003). Finally, in addition to physical health complications, circadian disruption is also associated with mental health complications, such as depression, anxiety and aggression (Barband & Nolan, 2008; Karl, Burne, & Herzog, 2006; Wersinger, Caldwell, Christiansen, & Young, 2007; Zelinksi et al., 2014). The complex relationship between mental wellbeing and circadian rhythmicity is further evidenced by research indicating that medicating depression reduces the symptoms of

circadian disruption, and that regulating circadian disruption alleviates symptoms of depression (Zelinski et al., 2014).

Due to today's societal advancements, the prevalence of circadian rhythm disruption is continually increasing, which in turn increases the comorbid health complications that are associated with such disruptions (Potter et al., 2016). It is, therefore, imperative to further understand the source of circadian rhythm disruption within our society. The two major sources of circadian disruption discussed throughout the literature are jet lag and shift work. Both jet lag and shift work have been shown to cause peripheral circadian oscillators to desynchronize from the SCN, subsequently leading to internal desynchronization (Barbard & Nolan, 2008).

Due to its strong association with cancer, shift work has been labelled a probable human carcinogen by the International Agency for Research on Cancer (IARC Report, 2010). Additionally, individuals experiencing shift work are more likely to have a higher body mass index (BMI) than non-shift workers, despite not consuming more daily calories (de Assis, Kupek, Vinicus-Nahas, & Bellisle, 2003; Di Lorenzo et al., 2003). This is indicative of the impact of shift work on metabolism, and more specifically on leptin and ghrelin production (de Assis et al., 2003). Furthermore, shift work has been associated with impaired glucose tolerance and subsequent increases in the likelihood of Type 2 Diabetes and increased blood pressure (Lund, Arendt, Hampton, English, & Morgan, 2001; Scheer, Hilton, Mantzoros, & Shea, 2009).

While valuable findings are discovered through correlational and epidemiological research investigating circadian rhythm disruption in human participants, it cannot be denied that such findings have the risk of being confounded by extraneous variables. To

account for such extraneous variables, while furthering the understanding of the impact of circadian disruption on learning, memory and physiology, numerous animal models of jet lag and shift work have been developed.

Sei et al. (2003) developed an animal model of jet lag, whereby rats were exposed to a single eight-hour phase advance, which involved the light phase of the LD cycle being shifted eight hours earlier (a photoperiod shift). They showed that hippocampal brain-derived neurotropic factor (BDNF), which is a neuroplasticity-related protein, was significantly increased the day following the phase shift. This indicates that acute jet lag can have physiological effects, impacting hippocampal metabolism. Similar increases in hippocampal BDNF are seen following ischemia, hypoglycemia, seizures and traumatic brain injuries, and therefore may be a neuroprotective factor (Binder, Croll, Gall, & Scharfman, 2001; Grundy, Patel, Harbuz, Lightman, & Sharples, 2000; Lindvall et al., 1992; Schmidt-Kastner et al., 2001).

Similar animal models have been created to mirror shift work, whereby the aforementioned photoperiod shifting (PPS) occurs, however for more than one shift. Devan et al. (2001) trained rats on a hippocampal-dependent Morris water maze (MWM) task (Morris, 1984) (MWM) while simultaneously exposing them to a three-hour phase advance everyday for six consecutive days, mimicking shift work in humans. While the rats' acquisition of the MWM was not impacted by the PPS, the shifted rats displayed significant retention impairment on the no-platform probe in comparison to control rats.

Likewise, Zelinski, Hong and McDonald (2014) trained rats on a hippocampal-dependent MWM and a non-hippocampal-dependent stimulus-response (SR) task, after which a PPS of three hours a day for six consecutive days was conducted. Following a

three-week period of circadian re-entrainment, the rats were tested on both tasks. The shifted rats displayed impaired retention on the hippocampal-dependent MWM, while retention on the non-hippocampal-dependent SR task was not affected. This demonstrated that the effects of PPS were not global effects, but rather localized and long lasting.

While the abundance of animal models of jet lag and shift work, along with the substantial epidemiological and correlational research on the topic, is fundamental to advance knowledge of circadian rhythms, there is a major gap in the literature. While jet lag and shift work do negatively impact circadian rhythmicity, there is another very common influencer on circadian rhythms that is often overlooked, called social jet lag. Social jet lag refers to the difference in an individual's biological timing and their social timing, or a discrepancy between an individual's sleep phase preference and their school or work schedule (Wittmann et al., 2006). The prevalence of social jet lag in society is staggering, with approximately 69 percent of adults experiencing at least one hour of social jet lag, and approximately 30 percent experiencing at least two hours of social jet lag on weekends (Roenneberg, Allebrandt, Merrow, & Vetter, 2012). Research indicates that social jet lag is associated with a wide variety of negative health outcomes, such as obesity, increased heart rate and increased cortisol levels (Kantermann et al., 2013; Parsons et al., 2015; Roenneberg et al., 2012; Rutters et al., 2014; Wong, Hasler, Kamarck, Muldoon, & Manuck, 2015). Additionally, social jet lag is associated with a two-fold increase in the risk of metabolic syndrome and diabetes, particularly in individuals under 61 (Koopman et al., 2017).

Individuals with an evening chronotype, meaning individuals who experience their period of optimal functioning later in the day, are more susceptible to social jet lag than morning chronotypes (Martin et al., 2016; Roenneberg et al., 2004). This is particularly concerning for the adolescent population, who tend to show much later chronotypes than other age groups (Roenneberg et al., 2004). Many adolescents are, therefore, required to function in the morning (due to early school start times), despite not having their optimal period of functioning until evening (Díaz-Morales, de León, & Sorroche, 2007). As a result, adolescents typically experience greater sleep insufficiency on school days compared to weekend days. Therefore, they often stay awake later on Friday and Saturday nights and sleep longer on Saturday and Sunday mornings, to make up for the past weeks' sleep insufficiency, which is characteristic of social jet lag (Martin et al., 2016). Consequently, adolescents with evening chronotypes are also more likely to perform poorly academically, and have a greater number of mental, behavioural and physical health implications compared to adolescents of other chronotypes (Gau et al., 2007; Preckel, Lipnevich, Schneider, & Roberts, 2011; Randler, 2011; Urban, Magyarodi, & Rigo, 2011). It is important to note that while adolescents are particularly susceptible to the negative effects of social jet lag, all individuals from primary school through to professional post-secondary school are susceptible to social jet lag if they display an evening chronotype (Smarr & Schirmer, 2018).

To date, only one animal model of social jet lag has been developed. Espitia-Bautista et al. (2017) placed rats in motorized running wheels for the first four hours of the light phase from Monday to Friday, to emulate societal requirements on humans during the work week. During the weekend the rats were not placed in the running

wheels. After combining their social jet lag paradigm with carbohydrate and fat-rich food (i.e., cafeteria diet), Espitia-Bautista et al. (2017) found that rats experiencing both social jet lag and cafeteria diet experienced five of the criteria for metabolic syndrome, including dyslipidemia and increased insulin levels.

The current studies propose a different animal model of social jet lag. Instead of using forced movement, as did Espitia-Bautista et al. (2017), the current model uses a lighting manipulation (see Table 1), similar to the animal models of jet lag and shift work previously discussed. The lighting manipulation used in the current study, which is named the Social Jet Lag Manipulation (SJM), was designed to mirror typical sleep habits characteristic of social jet lag, in an attempt to better understand its impact on learning and memory.

This study involved dividing rats based on their lighting exposure and FEO access. Rats were either exposed to the SJM, to experience social jet lag (SJM groups) or to the control (C) 12:12 LD cycle (C groups). Furthermore, the 1M groups received their total allotment of food in one meal per day, given at 1630. This allowed the 1M groups to have FEO access, due to mealtime regularity. In contrast, the MM groups received their total allotment of food in many meals given at random times throughout the light phase, at least two hours apart. This thereby prevented the MM groups from accessing their FEO, due to the inability of the FEO to synchronize to irregular mealtimes. Therefore, a total of four groups were investigated per experiment – 1M-SJM; MM-SJM; 1M-C and MM-C.

This study was composed of two experiments, the first of which examined the impact of LEO disruption elicited via social jet lag, and FEO access, on the retention of

hippocampal-dependent and non-hippocampal-dependent memory in rats. Rats were trained on a hippocampal-dependent water plus-maze (WPM) task and a non-hippocampal-dependent SR task and then exposed to one week of the SJM. Following SJM completion, retention testing occurred. Additionally, a context-dependent novel object recognition task (CDNOR) and an elevated plus-maze test (EPM) (to determine if any differences between groups were due to differences in stress) were conducted following the completion of the SJM.

The second experiment examined the impact of LEO disruption elicited via social jet lag, and FEO access, on the acquisition of hippocampal-dependent and non-hippocampal-dependent tasks in rats. Rats were trained on a hippocampal-dependent WPM task and a non-hippocampal-dependent SR task while simultaneously being exposed to the SJM. Additionally, a CDNOR and an EPM were conducted.

It was hypothesized that both acquisition and retention of hippocampal-dependent tasks would be negatively impacted by the experience of social jet lag, induced by the SJM. It was also hypothesized that acquisition and retention of non-hippocampal-dependent tasks would not be impacted by such experience. Furthermore, it was hypothesized that rats with FEO access would outperform rats without FEO access on all tasks, but particularly on hippocampal-dependent tasks. Specifically, it was hypothesized that the FEO would ameliorate the deleterious effects of the SJM, such that 1M-SJM rats would perform better than MM-SJM rats. Finally, it was hypothesized that rats in the 1M-C group would perform the best in both experiments, while the MM-SJM group would display the greatest impairments.

Experiment One: Retention

To assess the impact of social jet lag on retention, rats were first trained on hippocampal-dependent and non-hippocampal-dependent tasks, then exposed to one week of the SJM or Control (12:12) lighting before testing (Figure 1). To assess whether the FEO had an ameliorative effect on retention, access to the FEO was either established (by providing one meal per day) or prevented (by providing many meals per day).

Method

Subjects

Thirty-two male Long Evans rats (approximately 250 g at the start of training) were obtained from Charles River Laboratories (QC, Canada). Upon arrival, rats were singly housed in individually ventilated cages (32 cm x 35 cm x 18 cm) containing corncob bedding (Netco, New York, NY), Crink-l'Nest (The Anderson, Maumee, Ohio), a Nylabone (Nylabone Products, Neptune, NL), a wooden block and a piece of plastic pipe for enrichment. Shortly after arrival, one rat died unexpectedly, leaving a total of 31 rats.

After being housed in individually ventilated cages for one week, rats were transferred to individual cages (40 cm x 18 cm x 17 cm) attached to running wheels (36 cm in diameter). Each running wheel cage contained corncob bedding (Netco, New York, NY), Crink-l'Nest (The Anderson, Maumee, Ohio), a Nylabone (Nylabone Products, Neptune, NL), a wooden block and a piece of plastic pipe for enrichment. Additionally, each running wheel was connected to a computer system that continuously monitored wheel rotations. All rats were transferred to clear plastic conventional cages (45 cm x 25 cm x 21cm) with metal lids for transportation and testing.

For the first week after arrival, rats had *ad libitum* access to water and standard rat food (PMI Nutrition International, St. Louis, MO). After one week, rats were placed on a restricted feeding regime, allowing for a weight gain of 10g per week. Rats were maintained on a 12:12 light-dark cycle (lights on 0700-1900), in temperature-controlled rooms, prior to the onset of lighting manipulations.

Rats were randomly assigned to one of four groups, based on meal and lighting conditions. Rats in the 1M-SJM group (n=8) received their total allotment of food in one meal (given at 1630 daily, thereby allowing access to the FEO) and exposed to the SJM. Rats in the MM-SJM group (n=8) received their total allotment of food in many meals throughout the day (typically two to three meals per day at random times throughout the light cycle, thereby preventing access to the FEO) and exposed to the SJM. Rats in the 1M-C group (n=8) received their total allotment of food in one meal and were maintained on a 12:12 LD cycle for the duration of the experiment. Finally, rats in the MM-C group (n=7) received their total allotment of food in many meals throughout the day and were maintained on a 12:12 LD cycle. All meals were given during the light phase of the LD cycle, at least two hours apart (for days when multiple meals were given to the MM groups). Behavioural task training began three weeks after the onset of the restricted feeding regime, to allow sufficient time for the FEO to entrain.

All procedures used in the present experiment were conducted in accordance with the Canadian Council of Animal Care Guidelines and were approved by the Memorial University's Institutional Committee on Animal Care.

Apparatus

Stimulus-Response (SR) Maze. This apparatus consisted of a wooden plus maze that was painted white, and elevated 75 cm off the ground. Each arm of the plus maze was 15 cm wide and 53 cm long. At the end of each arm was an indented food cup, 0.5 cm deep. Fruity Whirls (Wal-Mart, Canada Corporation) were used as reinforcement and placed in these food cups. To prevent the use of olfactory cues during training, nylons filled with Fruity Whirls were taped to the underside of each arm. The stimulus of this stimulus-response task was a wire mesh that was placed around the correct arm, indicating the presence of a Fruity Whirl in the food cup.

The training room (617 cm x 358 cm) had several salient cues, including windows, three doors, a sink, a coat rack, a table and cupboards. During training, the rats' cages were aligned in chronological order along a countertop.

Context-Dependent Novel Object Recognition Arenas. This task involved the use of two open field arenas (92 cm x 92 cm x 123 cm), one painted black (Context 1) and the other painted white (Context 2). Both arenas were made of wood and placed on wheels.

A variety of objects were collected for this task, such as candleholders, figurines, and toys. All objects were difficult for rats to chew, easy to clean and part of an identical set of two. Objects were attached to the arenas using Velcro.

To ensure that all objects were equally appealing to rats, a pilot test was conducted whereby exploration time of all objects was observed using rats that had completed another experiment. During this pilot, the exploration time (defined as touching or smelling an object) for each object was recorded, and any objects that rats

spent greatly above or below average time exploring were not used in the task. This task took place in the same room as the SR task.

Water Plus- Maze (WPM). This apparatus consisted of a clear Plexiglas plus-maze inserted into a circular metal tank (120 cm in diameter x 29 cm deep) placed on a metal frame with wheels. The arms of the plus-maze insert were 11.5 cm wide and 52.5 cm long. The water level was maintained at 2 cm above the escape platform (located in one of the four arms), at room temperature (20-22 °C). The escape platform (11.5 cm in diameter x 26.5 cm high) was constructed from white PVC pipe filled with sand, attached to a Plexiglas base. The top of the escape platform was covered with white non-slip drawer liner. The water was made opaque by adding 250 mL of non-toxic white Tempera paint (Rich Art Color Company, Northvale, NJ).

The room (676 cm x 503 cm) had numerous salient cues that remained consistent throughout the duration of training, such as windows, a door, several tables, a computer, and posters. During training and testing rats were transferred into clear conventional cages lined with paper towel and placed in chronological order along a countertop within the room.

Elevated Plus Maze (EPM). This apparatus was made of wood and painted grey. The maze was elevated 75 cm off the floor and contained two “open arms” (i.e., arms containing no walls; 15.2 cm x 121.9 cm) and two “closed arms” (i.e., arms containing two enclosing walls and an open roof; 15.2 cm x 121.9 cm x 50.3 cm). This task took place in the same room as the SR task and the CDNOR task.

Procedure

Upon arrival, rats were given a three-week period to adjust to the 12:12 LD cycle and restricted feeding regime. Rats were then trained on the SR task and habituated to the CDNOR task, simultaneously. Following completion of CDNOR habituation, WPM training began. Once all training was complete, rats in the 1M-SJM and MM-SJM groups were exposed to one week of the SJM, while rats in the 1M-C and MM-C groups were maintained on a 12:12 LD cycle. Rats were not trained or tested during the lighting manipulation week. Following the completion of the lighting manipulation, rats were returned to a 12:12 LD cycle, and testing occurred. On the first day of testing, rats were examined on an EPM. Rats were also given a no-platform probe for the WPM task, five SR trials and a reminder session in both contexts of the CDNOR task. On the second test day, the CDNOR task was conducted.

Stimulus-Response (SR) Task. Each rat received two training sessions (of four trials each) per day for ten days, followed by one session per day for four days. Upon reaching criterion (18 out of 20 correct first arm entries), rats were removed from the task. Three rats did not meet criterion (all from the 1M groups), with a maximum of 100 trials given.

Rats were transported to the testing room in groups of eight (rats from groups 1M-C, 1M-SJM, MM-C and MM-SJM were included within each group of eight). Once in the training room, rats were organized in chronological order along a counter. Lights were on and a radio was playing during both training and testing.

During training, wire mesh was placed around a pseudorandomly chosen arm and half a Fruity Whirl was placed in the corresponding food cup. A rat was then placed on a

pseudorandomly chosen start arm, facing the experimenter. Latency to reach the Fruity Whirl was recorded. Rats were considered to have made a choice when their entire body minus their tail entered an arm. A trial was considered correct if a rat entered the mesh-covered arm on their first arm entry. For the first 20 trials rats were permitted to make incorrect choices, however they were removed after 120 seconds, if the Fruity Whirl was not discovered. After 20 trials, the rats were removed upon making an incorrect first choice.

A test trial was conducted eight days after the last training trial (i.e., one day after the completion of the lighting manipulation), in which all conditions were normal and the rats' latency to reach the Fruity Whirl and first arm choice were recorded. Four additional trials were subsequently conducted.

Context-Dependent Novel Object Recognition (CDNOR) Task. This task involved two phases, a habituation phase and a testing phase. The testing phase involved two exposure trials and one test trial. All trials were recorded using an overhead camera, and a blind experimenter coded object exploration time. Procedures for this task modeled those by Eacott and Norman (2004).

Habituation Phase. During this phase, rats were habituated to both contexts equally. Each rat received one habituation session per day, for eight days. A total of four habituation sessions were conducted in Context 1 (black arena) and four habituation sessions were conducted in Context 2 (white arena). Context habituation sessions were alternated daily and counterbalanced between groups. The first habituation session per context lasted 30 minutes, while all subsequent habituation sessions lasted 10 minutes. During each habituation session a random object was placed in the center of the area, to

ensure the rats were accustomed to objects being in the arena with them prior to the testing phase. These objects were not used during testing. All contexts and objects were cleaned with a 10% ethanol solution in between rats. Lights were on and a radio played during both habituation and testing phases.

During habituation sessions, rats were transported to the testing room in groups of eight. All rats were aligned in chronological order along the counter. Rats were placed within the arena, facing the south wall and permitted to explore freely.

Testing Phase. The testing phase occurred 18 days after the final habituation session (following the completion of lighting manipulations). As a precaution due to the extended period of time between habituation and testing, rats were given a reminder habituation session in each context, for 5 minutes each, the day prior to testing.

Testing sessions consisted of two exposure trials, followed by a test phase. Each exposure phase lasted five minutes, while the test phase lasted three minutes. There was a two-minute delay between phases, which allowed the experimenters time to clean all contexts and objects, and switch contexts and objects for the next phase. During the first exposure phase, either Context 1 (black) or Context 2 (white) was used (counterbalanced between groups). Within the context were two novel objects (Object A, a square candle holder and Object B, a metal egg holder). The placement of these objects (left/right) was counterbalanced between rats. Objects were secured to the context floor using Velcro and were equidistant (30 cm) from the walls. During the second exposure phase, the context that was not used in the first phase was now used. Additionally, the placement of the objects was switched, such that the object on the right during the first exposure phase was on the left during the second. During the testing phase, the context used in the first

exposure phase was reused. Two identical copies of either Object A or Object B (counterbalanced between rats) were used during this phase. The testing session process is outlined in Figure 2.

During all phases rats were placed into the context facing the south wall and permitted to explore freely for the duration of the phase. In between phases, during the two-minute delay, rats were returned to their holding cage while experimenters cleaned the contexts and objects and prepared the next phase.

Water Plus-Maze. Each rat received one session of 10 trials per day, for six consecutive days.

Rats were transported to the testing room in groups of eight. Once in the testing room, rats were transferred to clean conventional cages lined with paper towel and placed in chronological order along the counter. Lights were on and a radio was playing during both training and testing.

Training. Each rat was assigned a platform location pseudorandomly, such that all arms of the inserted water plus maze contained the platform an equal number of times, counterbalanced among rats. The platform location remained consistent per rat for the duration of training, with only the release positions changing. During each trial, rats were carried in their holding cage to their release position in a counterclockwise direction. The rat was then placed in the start arm, facing the experimenter. Latency to reach the platform and arm entries were recorded. If the rat did not reach the platform within 60 seconds, they were manually guided to the platform location, on which they remained for 10 seconds. The rat was then placed back into its holding cage and carried back to the counter in a clockwise direction. During all trials the experimenter remained standing at

the release location, while another experimenter stood in a fixed location away from the maze, recording arm entries. An entry was defined as the rat's entire body minus their tail entering an arm. A trial was considered correct if the rat entered the arm containing the platform on their first arm choice.

Testing. Testing occurred eight days after the last training day (following the completion of the lighting manipulation). During testing, each rat received one no-platform probe followed by five relearning trials. During the no-platform probe, the rat was released from a random start arm that was different than their start arm on their last training day. Rats were permitted to swim freely for 60 seconds. The no-platform probes were video recorded and arm entries, as well as the duration of time spent in each arm, was coded by a blind experimenter.

Elevated Plus-Maze. Each rat received one trial of the elevated plus maze test, which lasted for five minutes. The EPM test was conducted the day following the completion of the lighting manipulation.

Rats were transported to the testing room in groups of eight and placed in chronological order along the counter. Rats remained in their cages for a 30-minute acclimation period before the test began. Lights were on in the room during this test.

Following the acclimation period, each rat received one trial. The rat was placed in the center of the EPM, facing an open arm and permitted to explore for five minutes. A rat was considered to have entered an arm when its entire body, minus its tail was in an arm. Between each rat the maze was cleaned with a 10% ethanol solution. Trials were video recorded and a blind experimenter coded time spent in open and closed arms.

Results

Stimulus-Response Task

The stimulus-response task examined non-hippocampal-dependent retention. Figure 3 displays the average trials to criterion on the SR task for each group. Criterion was set at 18 out of 20 trials correct, after which a rat was removed from the task. If a rat did not reach criterion, their total number of completed trials was used in the analysis. An Analysis of Variance (ANOVA) indicated that there was no main effect of meal group (1M groups: M = 63.063, SD = 21.635; MM groups: M = 63.867, SD = 16.591), $F(1, 27) = .015$, $p = .904$, partial $\eta^2 = .001$. There were also no main effect of lighting condition (SJM groups: M = 63.688, SD = 21.275; Control groups: M = 63.200, SD = 17.089), $F(1,27) = .003$, $p = .959$, partial $\eta^2 < .0005$, which was to be expected as the lighting manipulation occurred following training. Likewise, there was no interaction, $F(1,27) = .120$, $p = .731$, partial $\eta^2 = .004$.

An ANOVA also revealed that there was no main effect of meal group for the percentage of first choice correct (1M groups: M = .938, SD = .250; MM groups: M = .933, SD = .258) on the first trial following the lighting manipulations, $F(1,27) = .000$, $p = 1.000$, partial $\eta^2 < .0005$. Similarly, there was no main effect of lighting condition (SJM groups: M = .875, SD = .342; Control groups: M = 1.00, SD = .000), $F(1,27) = 1.862$, $p = .184$, partial $\eta^2 = .065$, and no interaction, $F(1,27) = .000$, $p = 1.000$, partial $\eta^2 < .0005$. Therefore, there were no differences between all groups on the SR task, as hypothesized.

Context-Dependent Novel Object Recognition Task

The CDNOR task examined hippocampal-dependent memory. Double-blind experimenters coded all test phase videos for exploration time of each object. Exploration

was defined as the rat actively touching or smelling an object. Neither using the object as support during rearing, nor sitting on the object, were considered exploratory behaviours. A novelty ratio was calculated for each rat by comparing the time spent exploring the object with the novel object-place-context combination to the total exploration time. The average novelty ratios for each group are displayed in Figure 4.

An ANOVA indicated that there was no main effect of meal group on novelty ratios (1M groups: $M = .437$, $SD = .171$; MM groups: $M = .358$, $SD = .241$), $F(1,25) = 1.009$, $p = .325$, partial $\eta^2 = .039$. There was also no main effect of lighting condition (SJM groups: $M = .408$, $SD = .221$; Control groups: $M = .396$, $SD = .195$), $F(1,25) = .040$, $p = .842$, partial $\eta^2 = .002$. Likewise, there was no interaction, $F(1,25) = .028$, $p = .869$, partial $\eta^2 = .001$. These results suggest that, contrary to what was hypothesized, no differences in hippocampal-dependent memory, as measured by the CDNOR, existed between groups.

Water Plus-Maze Task

The WPM task examined hippocampal-dependent memory. Figure 5 displays the average trials to criterion on the WPM task for each group. Criterion was set at 18 out of 20 trials correct, with all rats reaching criterion. An ANOVA revealed that there was no main effect of meal group for trials to criterion (1M groups: $M = 36.750$, $SD = 13.429$; MM groups: $M = 30.270$, $SD = 10.159$), $F(1,27) = 2.525$, $p = .124$, partial $\eta^2 = .086$. There were also no main effect of light condition (SJM groups: $M = 37.190$, $SD = 12.194$; Control groups: $M = 29.800$, $SD = 11.409$), $F(1,27) = 3.233$, $p = .083$, partial $\eta^2 = .107$, which was to be expected as this training occurred prior to the lighting manipulation onset. Furthermore, there was no interaction, $F(1,27) = .349$, $p = .560$, partial $\eta^2 = .013$.

Double-blind experimenters coded each 60-second probe trial for first arm entries and total time in the target and other arms. An arm entry was defined as the rat's entire body, minus their tail, entering an arm. Figure 6 displays the percentage of 'first arm choice correct' for each group. An ANOVA indicated that there was no main effect of meal group on the percentage of first arm choice correct (1M groups: $M = .750$, $SD = .447$; MM groups: $M = .730$, $SD = .458$), $F(1,27) = .011$, $p = .916$, partial $\eta^2 < .0005$, nor was there a main effect of lighting (SJM groups: $M = .750$, $SD = .447$; Control groups: $M = .730$, $SD = .458$), $F(1,27) = .011$, $p = .916$, partial $\eta^2 < .0005$. Likewise, there was no interaction, $F(1,27) = .011$, $p = .916$, partial $\eta^2 < .0005$.

Figure 7A displays the average time each group spent in the target arm during the 60-second probe. An ANOVA revealed that there was no main effect of meal group (1M groups: $M = 14.720$, $SD = 4.982$; MM groups: $M = 13.450$, $SD = 4.140$), $F(1,27) = .654$, $p = .426$, partial $\eta^2 = .024$, nor was there a main effect of lighting condition (SJM groups: $M = 13.830$, $SD = 4.312$; Control groups: $M = 14.390$, $SD = 4.952$), $F(1,27) = .071$, $p = .792$, partial $\eta^2 = .003$. Likewise, there was no interaction, $F(1,27) = 1.979$, $p = .171$, partial $\eta^2 = .068$.

The average time each group spent in the incorrect arms during the 60-second probe is displayed in Figure 7B. This time was calculated by dividing the total time spent in all three incorrect arms by three, to obtain the average time the rats spent in each incorrect arm. An ANOVA indicated that there was a main effect of meal group, with the 1M groups ($M = 10.330$, $SD = 1.146$) spending significantly less time in the incorrect arms than the MM groups ($M = 11.360$, $SD = 1.631$), $F(1,27) = 4.412$, $p = .045$, partial $\eta^2 = .140$. However, there was no main effect of lighting condition (SJM groups: $M =$

10.940, *SD* = 1.464; Control groups: *M* = 10.710, *SD* = 1.528), *F*(1,27) = .122, *p* = .730, partial η^2 = .005, nor was there an interaction, *F*(1,27) = 2.135, *p* = .156, partial η^2 = .073.

Therefore, these results suggest that, as hypothesized, rats with access to their FEO (i.e., rats within the 1M groups) perform better on hippocampal-dependent tasks, as measured by the WPM.

Elevated-Plus Maze

The EPM examines differences in anxiety-like behaviour among groups. The average times spent in the open and closed arms of the EPM for each group are displayed in Figure 8.

An ANOVA revealed that there was no main effect of meal groups on time spent in open arms (1M groups: *M* = 49.575, *SD* = 25.749; MM groups: *M* = 58.793, *SD* = 35.902), *F*(1,27) = .627, *p* = .436, partial η^2 = .023. Similarly, there was no main effect of lighting condition (SJM groups: *M* = 55.340, *SD* = 36.732; Control groups: *M* = 52.813, *SD* = 25.442), *F*(1,27) = .053, *p* = .820, partial η^2 = .002, and no interaction, *F*(1,27) = .237, *p* = .631, partial η^2 = .009.

Likewise, an ANOVA revealed that there was no main effect of meal group on time spend in the enclosed arms (1M groups: *M* = 109.056, *SD* = 29.201; MM groups: *M* = 101.307, *SD* = 37.445), *F*(1,27) = .403, *p* = .531, partial η^2 = .015. There were also no main effect of lighting condition (SJM groups: *M* = 101.067, *SD* = 32.266; Control groups: *M* = 109.281, *SD* = 34.437), *F*(1,27) = .451, *p* = .507, partial η^2 = .016, and no interaction, *F*(1,27) = .273, *p* = .606, partial η^2 = .010. Therefore, these results suggest that no differences in stress or anxiety-like behaviour, as measured by the EPM, existed between groups.

Running Wheels

To examine the circadian rhythmicity of rats, activity levels are typically examined via running wheels. However, formal analysis of the running wheel data for the current experiment was not possible, as rats were removed from running wheel cages during the day for training. Rats were typically removed from their running wheel cages in the morning and placed in conventional cages for training, then returned in the evening. The actograms produced were, therefore, not a valid representation of the total activity of the rats, as they were also active while removed from the running wheel cages. As such, a formal analysis of the running wheel data would produce invalid information regarding circadian rhythmicity, due to missing daytime data. Instead, visual observations of the actograms were made, to compare differences between groups, as all groups were removed from their running wheels for the same amount of time (Figures 9, 10, 11 and 12).

Discussion

Experiment One investigated the impact of the SJM, in addition to the impact of FEO access, on retention of hippocampal-dependent (WPM and CDNOR) and non-hippocampal-dependent (SR) tasks. It was hypothesized that rats exposed to the SJM would exhibit retention deficits on the hippocampal-dependent tasks but not on the non-hippocampal-dependent task. It was also hypothesized that rats with access to their FEO would outperform rats without FEO access. Additionally, it was hypothesized that rats experiencing the SJM would exhibit more anxiety-like behaviour. Finally, it was hypothesized that the FEO would ameliorate the deleterious effects of the SJM.

The results from the current study did not support the hypothesis that the SJM would impact retention of hippocampal-dependent tasks. The SJM groups displayed no retention deficits on the WPM or CDNOR tasks in comparison to the Control groups. This contradicts previous studies, which found retention deficits on hippocampal-dependent tasks following one week of lighting manipulations modeling shift work (Zelinski et al., 2014). Additionally, contrary to what was hypothesized, the results from the current study suggest that anxiety-like behaviour between groups did not differ.

Furthermore, the results supported the hypothesis that the SJM would not impact retention of non-hippocampal-dependent tasks, as both SJM and Control groups performed equally on the SR task. This supports previous findings, which also found no retention deficits on non-hippocampal-dependent tasks when training was followed by one week of lighting manipulations emulating shift work (Zelinski et al., 2014).

Additionally, the hypothesis that FEO access would benefit retention was supported by the current study. Specifically, rats without access to their FEO spent significantly more time in the incorrect arms of the WPM during the probe trial than rats with FEO access. This suggests that rats with FEO access had more definitive knowledge of the platform location, resulting is less exploration of the incorrect arms compared to rats without FEO access. This finding is additive to the current body of literature, as FEO access has been shown to improve TPL, but has not been investigated with other tasks (Wall et al., 2018). The current study demonstrated that FEO access is beneficial for retention of hippocampal-dependent tasks, while retention of non-hippocampal tasks does not appear to be improved by FEO access.

Unfortunately, because there were no retention impairments produced by the SJM, the ameliorative effects of FEO access could not be investigated in the current study. Therefore, the hypothesis that FEO access would ameliorate the deleterious effect of the SJM could not be investigated.

Experiment Two: Acquisition

The results from Experiment One suggest that the SJM did not appear to have an effect on retention of either hippocampal-dependent or non-hippocampal-dependent tasks. However, rats with FEO access performed better than their counterparts without FEO access on the WPM probes. Based on these results, it was decided that the impact of social jet lag on acquisition should also be investigated. As such, rats were trained on both hippocampal-dependent and non-hippocampal-dependent tasks while simultaneously being exposed to either the SJM or Control (12:12) lighting (Figure 13). Additionally, to assess whether the FEO had an ameliorative effect on acquisition, access to the FEO was either established (by providing one meal per day) or prevented (by providing many meals per day).

Method

Subjects

Thirty-two male Long Evans rats (approximately 300 g at the start of training) were obtained from another researcher within the same research facility. The rats were originally obtained from Charles River Laboratories (QC, Canada). Prior to transfer, the rats had been trained on a WPM task that investigated directional learning. Due to differences in the research paradigms and task procedures, it was believed that such previous experience would not negatively impact this experiment.

Upon transfer, rats were singly housed in individually ventilated cages (32 cm x 35 cm x 18 cm) containing corncob bedding (Netco, New York, NY), Crink-l'Nest (The Anderson, Maumee, Ohio), a Nylabone (Nylabone Products, Neptune, NL), a wooden block and a piece of plastic pipe for enrichment. Rats were placed on a restricted feeding regime allowing for a weight gain of 10g per week and maintained on a 12:12 light-dark cycle (lights on 0700-1900), in temperature controlled rooms, prior to the onset of lighting manipulations.

Rats were randomly assigned to the same groups (n=8 per group) previously described in Experiment One. Due to time constraints, rats were run in two separate cohorts. The first cohort consisted of all MM rats, as they did not require time to entrain to meal times, as did the 1M groups. Following three weeks on the restricted feeding regime, training for the 1M rats began.

Similarly, due to these time constraints rats were not housed in cages attached to running wheels during Experiment Two. It is necessary to provide rats with a period of habituation once housed in cages attached to running wheels, prior to the onset of behavioural testing. Such period of habituation was not possible during this Experiment. As such, it was not possible to compare behaviour between experiments due to the potential effects of physical activity on cognition (reference). This was considered acceptable for the current study, however, as comparisons were already impossible due to other potential confounds (i.e. rats having participated in a previous experiment). Additionally, each experiment was assessing a different cognitive factor (retention versus acquisition), meaning comparison between experiments was not necessary.

All procedures used in the present experiment were conducted in accordance with the Canadian Council of Animal Care Guidelines and were approved by the Memorial University Institutional Committee on Animal Care.

Apparatus

Each testing apparatus used in Experiment Two was reused from Experiment One and are described above.

Procedure

Rats were trained on the WPM, SR task and habituated to the CDNOR task, simultaneously. Rats in the 1M-SJM and MM-SJM groups were exposed to the SJM throughout the entirety of training (with SJM onset occurring five days prior to training onset). All training was conducted during the light phase, at the same time each day whenever possible. Following the completion of training, the lighting manipulations ceased and rats were examined on an EPM. Additionally, a CDNOR task was conducted.

Four days following the completion of training, rats received a massed water plus-maze (MWPM) training session. A no-platform probe was conducted 24 hours later. Two weeks following the first MWPM training session, a second session was conducted, along with a no-platform probe 24 hours later.

Stimulus-Response (SR) Task. Each rat received one training session (of eight trials) per day for 15 days. The procedure for this SR task was identical to the SR procedure outlined in Experiment One.

Context-Dependent Novel Object Recognition Task. The procedure for this CDNOR task was identical to the CDNOR procedure outlined in Experiment One.

Water Plus-Maze Task. Each rat received one session of five trials per day, for 15 days. The procedure for this WPM task was identical to the WPM task procedure outlined in Experiment One.

Massed Water Plus-Maze Task. Four days following the completion of WPM training, rats underwent a MWPM session whereby they received one session of 10 trials. During this training session the rats' target arm was different than during the previous WPM task. Various cues within the room (i.e., curtains, posters, etc.) were moved to ensure a contextual change in relation to the previous WPM task. All other procedures remained the same. Twenty-four hours after training, a single no-platform probe was conducted. Each probe was video recorded and coded by a blind experimenter.

Two weeks following the first MWPM task, a second MWPM task was conducted. During this task, the rats were assigned to a new target arm (different than their previous two target arm assignments). The procedure was identical to the first MWPM task, with a no-platform probe being conducted 24 hours later. Each probe was video recorded and coded by a blind experimenter.

Elevated Plus-Maze. Following the completion of WPM, SR and CDNOR training (i.e. the day following the completion of the lighting manipulation), each rat was placed on the EPM for five minutes. The procedure for this EPM is identical to the EPM procedure outlined in Experiment One.

Results

Stimulus-Response Task

The stimulus-response task examined non-hippocampal-dependent learning. The data were grouped into 10 blocks of six trials to analyze acquisition of the SR task.

Acquisition for each group is displayed in Figure 14. A between-within ANOVA revealed that there was a significant quadratic effect of Block, $F(1,28) = 4.543$, $p = .042$, partial $\eta^2 = .140$, indicating that all groups were learning over time. There were, however, no differences in acquisition between the 1M and MM groups, $F(1,28) = .010$, $p = .922$, partial $\eta^2 < .0005$, nor between the SJM and Control groups, $F(1,28) = .416$, $p = .524$, partial $\eta^2 = .015$. Additionally, there was no interaction, $F(1,28) = .298$, $p = .589$, partial $\eta^2 = .011$.

Trials to criterion on the SR task were also examined, and the average trials to criterion for each group are displayed in Figure 15. Criterion was set at 18 out of 20 trials correct, with only six rats reaching criterion (two rats from the MM-SJM group; one rat from the MM-C group; and three rats from the 1M-SJM group). If a rat did not reach criterion, their total number of completed trials was used in the analysis (maximum of 60 trials). An ANOVA indicated that there were was no main effect of meal group (1M groups: $M = 58.060$, $SD = 5.092$; MM groups: $M = 59.000$, $SD = 2.556$), $F(1, 28) = .461$, $p = .503$, partial $\eta^2 = .016$. There was also no main effect of lighting condition (SJM groups: $M = 57.560$, $SD = 5.189$; Control groups: $M = 59.500$, $SD = 2.000$), $F(1,28) = 1.970$, $p = .171$, partial $\eta^2 = .066$. Likewise, there was no interaction, $F(1,28) = 1.970$, $p = .171$, partial $\eta^2 = .066$. Therefore, there were no differences between all groups on the SR task, as hypothesized.

Context-Dependent Novel Object Recognition Task

The CDNOR task examined hippocampal-dependent memory. Double-blind experimenters coded all test phase videos for exploration time of each object, which was defined as active touching or smelling of the object. Neither using the object as support

during rearing, nor sitting on the object, were considered exploratory behaviours. A novelty ratio was calculated for each rat by comparing the time spent exploring the object with the novel object-place-context combination to the total exploration time. The average novelty ratios for each group are displayed in Figure 16.

An ANOVA indicated that there was no main effect of meal group in the novelty ratios (1M groups: $M = .6109$, $SD = .238$; MM groups: $M = .632$, $SD = .174$), $F(1,28) = .076$, $p = .785$, partial $\eta^2 = .003$. There were also no main effect of lighting condition (SJM groups: $M = .617$, $SD = .207$; Control groups: $M = .625$, $SD = .211$), $F(1,28) = .011$, $p = .916$, partial $\eta^2 < .0005$. Likewise, there was no interaction, $F(1,28) = .993$, $p = .328$, partial $\eta^2 = .034$. These results suggest that, contrary to what was hypothesized, no differences in hippocampal-dependent learning, as measured by the CDNOR, existed between groups.

Water Plus-Maze Task

The WPM task examined hippocampal-dependent learning. Data were grouped into 10 blocks of seven trials to analyze acquisition of the WPM task. Acquisition for each group is displayed in Figure 17. A between-within ANOVA revealed that there was a significant cubic effect of Block, $F(1,28) = 10.156$, $p = .004$, partial $\eta^2 = .266$, indicating that all groups were learning over time. There was also a significant difference in acquisition between the 1M and MM groups, $F(1,28) = 6.754$, $p = .015$, partial $\eta^2 = .194$, indicating that the 1M groups ($M = 6.500$, $SD = .730$) learned the task faster than the MM groups ($M = 5.000$, $SD = 2.160$). There were, however, no differences between the SJM and Control groups, $F(1,28) = .050$, $p = .824$, partial $\eta^2 = .002$, and no interaction, $F(1,28) = .545$, $p = .467$, partial $\eta^2 = .019$.

Figure 18 displays the average trials to criterion on the WPM task for each group. Criterion was set at 18 out of 20 trials correct, with only one rat (from the 1M-C group) not reaching criterion. If a rat did not reach criterion, their total number of completed trials was used in the analysis (maximum of 75 trials). An ANOVA revealed that there was a significant main effect of meal group on trials to criterion (1M groups: $M = 24.733$, $SD = 6.692$; MM groups: $M = 33.438$, $SD = 14.710$), $F(1,27) = 4.297$, $p = .048$, partial $\eta^2 = .137$, indicating that the 1M groups required fewer trials to reach criterion compared to the MM groups. There was, however, no main effect of lighting condition (SJM groups: $M = 29.875$, $SD = 10.887$; Control groups: $M = 28.533$, $SD = 13.804$), $F(1,27) = .163$, $p = .690$, partial $\eta^2 = .006$. Furthermore, there was no interaction, $F(1,27) = .370$, $p = .548$, partial $\eta^2 = .014$.

Therefore, these results suggest that, as hypothesized, rats with access to their FEO (i.e., rats within the 1M groups) perform better on hippocampal-dependent tasks, as measured by the WPM.

Massed Water Plus-Maze

The MWPM further examined hippocampal-dependent learning. Double-blind experimenters coded each 60-second probe trial for first arm entries and total time in the target and other arms. An arm entry was defined as the rat's entire body, minus their tail, entering an arm.

Massed Water Plus-Maze Probe One. Figure 19 displays the percentage first arm choice correct for each group during the first 60-second probe following massed WPM training. An ANOVA indicated that there were no main effect of meal group on the percentage of first arm choice correct (1M groups: $M = .630$, $SD = .500$; MM groups:

M = .500, *SD* = .516), *F*(1,28) = .467, *p* = .500, partial η^2 = .016, nor was there a main effect of lighting condition (SJM groups: *M* = .630, *SD* = .500; Control groups: *M* = .500, *SD* = .516), *F*(1,28) = .467, *p* = .500, partial η^2 = .016. Likewise, there was no interaction, *F*(1,28) = .467, *p* = .500, partial η^2 = .016.

Figure 20A displays the average time each group spent in the target arm during the first probe following the massed WPM training. An ANOVA revealed that there was no main effect of meal group (1M groups: *M* = 16.730, *SD* = 3.494; MM groups: *M* = 14.630, *SD* = 4.884), *F*(1,27) = 1.717, *p* = .201, partial η^2 = .060, nor was there a main effect of lighting condition (SJM groups: *M* = 15.750, *SD* = 4.494; Control groups: *M* = 15.530, *SD* = 4.307), *F*(1,27) = .016, *p* = .901, partial η^2 = .001. Likewise, there was no interaction, *F*(1,27) = .852, *p* = .364, partial η^2 = .031.

The average time each group spent in an incorrect arm during the first probe following the massed WPM training is displayed in Figure 20B. This time was calculated by dividing the total time spent in all three incorrect arms by three, to obtain the average time the rats spent in each incorrect arm. An ANOVA indicated that there was no main effect of meal group (1M groups: *M* = 9.630, *SD* = 1.258; MM groups: *M* = 10.380, *SD* = 1.746), *F*(1,28) = 1.902, *p* = .179, partial η^2 = .064. There was also no main effect of lighting condition (SJM groups: *M* = 9.940, *SD* = 1.526; Control groups: *M* = 10.060, *SD* = 1.611), *F*(1,28) = .053, *p* = .820, partial η^2 = .002, nor was there an interaction, *F*(1,28) = 1.321, *p* = .260, partial η^2 = .045.

Massed Water Plus-Maze Probe Two. The percentage of first arm choice correct for each group during the second probe following the massed WPM training is displayed in Figure 21. An ANOVA indicated that there was a significant main effect of

meal group for the percentage of first arm choice correct (1M groups: $M = 1.000$, $SD = .000$; MM groups: $M = .688$, $SD = .479$), $F(1,28) = 6.481$, $p = .017$, partial $\eta^2 = .188$, such that the 1M groups made more correct first arm choices than the MM groups. There was, however, no main effect of lighting condition (SJM groups: $M = .875$, $SD = .342$; Control groups: $M = .813$, $SD = .403$), $F(1,28) = .259$, $p = .615$, partial $\eta^2 = .009$. Likewise, there was no interaction, $F(1,28) = .259$, $p = .615$, partial $\eta^2 = .009$.

Figure 22A displays the average time each group spent in the target arm during the second probe following the massed WPM training. An ANOVA revealed that there was no main effect of meal group (1M groups: $M = 15.625$, $SD = 4.500$; MM groups: $M = 13.688$, $SD = 4.512$), $F(1,28) = 1.384$, $p = .249$, partial $\eta^2 = .047$, nor was there a main effect of lighting condition (SJM groups: $M = 14.500$, $SD = 3.688$; Control groups: $M = 14.813$, $SD = 5.382$), $F(1,28) = .036$, $p = .851$, partial $\eta^2 = .001$. Furthermore, there was no interaction, $F(1,28) = .036$, $p = .851$, partial $\eta^2 = .001$.

The average time each group spent in an incorrect arm during the second probe following massed WPM training is displayed in Figure 22B. This time was calculated by dividing the total time spent in all three incorrect arms by three, to obtain the average time the rats spent in each incorrect arm. An ANOVA indicated that there was a significant main effect of meal group (1M groups: $M = 9.250$, $SD = 1.693$; MM groups: ($M = 10.375$, $SD = 1.310$), such that the 1M groups spent less time in the incorrect arms than the MM groups, $F(1,28) = 4.536$, $p = .042$, partial $\eta^2 = .139$. There was, however, no main effect of lighting condition (SJM groups: $M = 10.125$, $SD = 1.668$; Control groups: $M = 9.500$, $SD = 1.506$), $F(1,28) = 1.400$, $p = .247$, partial $\eta^2 = .048$, nor was there an interaction, $F(1,28) = 1.400$, $p = .247$, partial $\eta^2 = .048$.

Therefore, these results suggest that, as hypothesized, rats with access to their FEO (i.e., rats within the 1M groups) perform better on hippocampal-dependent tasks, as measured by the MWPM.

Elevated-Plus Maze

The EPM examines differences in anxiety-like behaviour among groups. The average time spent in the open and closed arms of the EPM for each group is displayed in Figure 23.

An ANOVA revealed that there was no main effect of meal group on the amount of time spent in open arms (1M groups: $M = 108.810$, $SD = 34.603$; MM groups: $M = 102.880$, $SD = 32.942$), $F(1,28) = .236$, $p = .631$, partial $\eta^2 = .008$. Similarly, there was no main effect of lighting condition (SJM groups: $M = 110.440$, $SD = 21.062$; Control groups: $M = 101.250$, $SD = 42.576$), $F(1,27) = .053$, $p = .820$, partial $\eta^2 = .020$, and no interaction, $F(1,28) = .565$, $p = .458$, partial $\eta^2 = .004$.

Likewise, an ANOVA revealed that there was no main effect of meal group on time spent in enclosed arms (1M groups: $M = 120.560$, $SD = 19.589$; MM groups: $M = 129.630$, $SD = 21.181$), $F(1,28) = 1.504$, $p = .230$, partial $\eta^2 = .051$. There was also no main effect of lighting condition (SJM groups: $M = 123.130$, $SD = 18.055$; Control groups: $M = 127.060$, $SD = 23.279$), $F(1,28) = .284$, $p = .598$, partial $\eta^2 = .010$, and no interaction, $F(1,28) = .302$, $p = .587$, partial $\eta^2 = .011$. Therefore, these results suggest that no differences in stress or anxiety-like behaviour, as measured by the EPM, existed between groups.

Discussion

Experiment Two investigated the impact of the SJM, in addition to the impact of FEO access, on acquisition of hippocampal-dependent (WPM, MWPM and CDNOR) and non-hippocampal-dependent (SR) tasks. It was hypothesized that rats exposed to the SJM would exhibit acquisition deficits on the hippocampal-dependent tasks but not on non-hippocampal-dependent task. It was also hypothesized that rats with FEO access would outperform rats without FEO access. Furthermore, it was hypothesized that the FEO would ameliorate the deleterious effects of the SJM.

The results from the current study did not support the hypothesis that the SJM would negatively impact acquisition of hippocampal-dependent tasks. The current study found that SJM and Control groups performed equally on the WPM, MWPM and CDNOR tasks. These findings contradict previous research, which found that acquiring a task while simultaneously experiencing lighting manipulations modeling shift work resulted in retention deficits in rats (Devan et al., 2001). More specifically, rats trained on a MWM while experiencing lighting manipulations displayed retention deficits during probe trials, which was not found in the current study. Likewise, all groups displayed similar behaviour on the EPM, suggesting that anxiety levels of all groups were similar. Furthermore, the hypothesis that the acquisition of non-hippocampal-dependent tasks would not be impacted by the SJM was supported, as the SJM and Control groups did not differ in their acquisition of the SR task.

As with Experiment One, the hypothesis that FEO access would be beneficial for acquisition was supported by the current study. Specifically, rats with FEO access acquired the WPM faster than rats without FEO access. Furthermore, during the second

probe following MWPM training, rats with access to their FEO displayed more correct first arm choices than rats without FEO access. Additionally, rats with FEO access spent less time in the incorrect arms during the second probe following massed training compared to rats without FEO access, indicating that they acquired a more definitive understanding of the platform location during acquisition. As with Experiment One, these findings support the notion that FEO access is beneficial for hippocampal-dependent tasks. Finally, due to the SJM not having deleterious effects on acquisition, the current study was not able to investigate whether FEO access was ameliorative. Therefore, the hypothesis that FEO access would be ameliorative to the deleterious effects of the SJM remains to be investigated.

General Discussion

The current study aimed to establish a novel animal model of social jet lag, similar to previously established animal models of shift work and jet lag. While an animal model of social jet lag has been established (Espitia-Bautista et al., 2017), it differs greatly from models of jet lag and shift work (Sei et al., 2003; Devan et al., 2001; Zelinski et al., 2014), due to its use of motorized running wheels to ensure wakefulness. The use of forced movement makes it difficult to compare the results found by Espitia-Bautista et al. (2017) to other studies investigating circadian disruptions in animal models, as other models solely use lighting manipulations to emulate circadian disruption. By establishing a non-invasive animal model of social jet lag that uses lighting manipulations only, such comparisons could be made.

As such, the SJM was developed to mirror social jet lag. Using the SJM paradigm, the current study investigated the implications of social jet lag on both

acquisition and retention of hippocampal-dependent (WPM and CDNOR) and non-hippocampal dependent (SR) tasks. The role of the FEO and its potential to ameliorate the negative effects of social jet lag, along with its potential to benefit learning and memory, were also investigated.

While previously established animal models of jet lag and shift work negatively impacted the acquisition and retention of hippocampal-dependent tasks, the SJM did not produce such findings. The lack of similar findings would, presumably, suggest that the SJM is not as impactful a model of circadian disruption as the aforementioned animal models of jet lag and shift work. This, however, may not be true. When examining the actograms (visual representations of circadian rhythmicity obtained via running wheels) of animals exposed to the shift work model implemented by Zelinski et al. (2014), free-running behaviour is clearly observed upon implementation of the lighting manipulation. Essentially, following implementation of the shift work lighting manipulation paradigm, the rats' onset of activity altered slightly each day, such that the onset of activity was delayed in comparison to the previous day, while the duration of activity remained the same. This pattern continued throughout the entirety of the lighting manipulation, thereby producing characteristic diagonal patterns of activity on the final actograms (see Figure 24). In addition to causing free-running activity, this lighting manipulation also negatively impacted the acquisition and retention of hippocampal-dependent tasks (Devan et al., 2001; Zelinski et al., 2014). If the SJM was not capable of negatively impacting the acquisition and retention of hippocampal-dependent tasks in this same manner, then one would assume that it must not have been capable of causing free-running activity either. This, however, is not true. Upon examination of the actograms of

rats exposed to the SJM, it is evident that the same free-running behaviour inflicted by the animal model of shift work (Zelinski et al., 2014) is also inflicted by the SJM (see Figures 11 and 12). This would suggest that the SJM was capable of disrupting circadian rhythms in the same manner as previously established animal models of circadian disruption.

There are several possible explanations as to why the SJM could produce the same free-running activity as other animal models of circadian disruption, yet not the same deficits on acquisition and retention of hippocampal-dependent tasks. The most likely explanation is that the testing apparatus used in the current study was not as sensitive to hippocampal impairments as those used in previous studies. More specifically, while studies modeling shift work used a MWM as a hippocampal-dependent task (Craig & McDonald, 2008; Devan et al., 2001; Zelinski, Tyndall, Hong, & McDonald, 2013; Zelinski et al., 2014), the current study used a WPM. The standard MWM is an open field and is, therefore, more difficult to acquire than a WPM, and therefore possibly more sensitive to hippocampal impairments.

A second possible explanation for why the SJM could produce the same free-running activity as other animal models, yet not the same deficits on hippocampal-dependent task acquisition and retention, is that the SJM possibly induced a different amount of stress than other established animal models of circadian disruption. Stress is known to have an impact on hippocampal-dependent learning and memory, since the hippocampus has the highest density of glucocorticoid receptors of all brain structures (McEwen, 2000; Mohammadi, Goudarzi, Lashkarbolouki, Abrari, & Salmani, 2014). While the current study examined rats on an EPM, many other studies modeling circadian

disruption (e.g. Zelinski et al., 2013; Zelinski et al., 2014) did not administer any stress measure. Therefore, while the SJM and Control rats in the current study did not have differing stress levels (interpreted by time spent in open arms of the EPM), it is possible that the rats receiving lighting manipulations in other studies were experiencing higher levels of stress than their control counterparts. Additionally, the SJM paradigm may be less drastic than other animal models of circadian rhythm disruption. For example, photoperiod shifting involves shifting the light onset time by three hours a day, everyday. In contrast, the SJM involves fairly consistent weekday light onset and offset times, with only the weekends having a more drastic difference in the timing of light onset and offset. Given that the SJM involves only two days per week with dramatic lighting manipulations, while photoperiod shifting involves daily dramatic lighting manipulations, it is very possible that the stress levels of animals experiencing photoperiod shifting would be greater in comparison to animals receiving the SJM. Therefore, the observed deficits in hippocampal-dependent task acquisition and retention observed in other studies could be an effect of stress, and not of circadian disruption directly. This would also explain why both the SJM rats and rats in previously discussed studies all exhibit free-running behaviour, a direct effect of circadian disruption, yet differences in acquisition and retention deficiencies.

While the current study did not find deleterious effects of the SJM, beneficial effects of FEO access were observed. More specifically, the current study demonstrated the benefits of FEO access on both acquisition and retention of hippocampal-dependent tasks. In both Experiment One and Experiment Two, rats with FEO access spent significantly less time in the incorrect arms of the WPM during probes, suggesting that

rats with FEO access had a better knowledge of the platform location. Furthermore, rats with FEO access acquired the WPM faster than rats without FEO access, and made more correct first arm choices following massed training. These findings contribute significantly to the literature, as thus far the beneficial effects of FEO access have only been studied in relation to TPL (Wall et al., 2018). The current study demonstrated that FEO access improved performance on hippocampal-dependent tasks, yet not performance on non-hippocampal-dependent tasks. This compliments previous research suggesting the hippocampus as a possible central anatomical location of the FEO (Munn, Tyree, McNaughton, & Bilkey, 2015). Additionally, previous research suggested that the hippocampus was particularly responsive to the FEO, compared to other brain areas (Munn et al., 2015). The current study supports this notion, as the non-hippocampal-dependent tasks, which rely on the dorsal striatum (Broadbent, Squire, & Clark, 2007), were not responsive to FEO access, while the hippocampal-dependent WPM and MWPM were.

The current study produced significant findings regarding the benefits of FEO access. To build upon such findings, future studies should ensure that rats from all groups are incorporated into each cohort. In the current study, Experiment Two was completed in two cohorts such that the first cohort contained only rats without FEO access (MM groups) and the second cohort contained only rats with FEO access (1M groups). Experiment Two was conducted in this manner due to time constraints, which were solved by investigating the MM rats first, as they did not require time for their FEOs to entrain to mealtimes. While the MM rats were being trained and tested, the 1M rats were given time to entrain to mealtimes, thereby allowing both cohorts to be tested in a shorter

period of time. However, we endeavored to make sure that both groups were trained in exactly the same way. Also, given that the results from Experiment Two were similar to those from Experiment One, it is less likely that these were spurious findings.

Additionally, future studies should investigate the current animal model of social jet lag using female rats. While using solely male rats avoids the confounds of distracting pheromones and estrus cycles, it also limits the applicability of findings. A major goal of animal models is to establish foundational knowledge, of which research involving human participants can build upon. However, while it was unfortunate that female rats were not included in the current study, previous research has demonstrated that female rats are less susceptible to circadian rhythm disruption than male rats (Zelinski et al., 2013). Therefore, it is unlikely that female rats would have exhibited deficits on the hippocampal-dependent tasks in response to the SJM, when the current male rats did not.

Furthermore, future studies should aim to train animals at varying times of day, as opposed to the same time each day. Previous research has found that attentionally demanding cognitive tasks can serve as zeitgebers, meaning that circadian rhythms can entrain to such cognitive tasks, if their timing is consistent (Gritton, Stasiak, Sarter, & Lee, 2012). If rats exposed to the SJM had disrupted circadian rhythms, it is possible that they began to entrain to the cognitive tasks, in an attempt to regain internal synchronization. This entrainment to the cognitive tasks could, thereby, mask the negative effects of the SJM. Future research should investigate whether rats are likely to entrain to attentionally demanding cognitive tasks, especially when information from their lighting environment is unreliable, such as during the SJM. Finally, future studies

should utilize a MWM in place of the currently used WPM, to ensure sufficient sensitivity to display any hippocampal deficiencies.

Given the prevalence of social jet lag in today's society, it is imperative to continue researching this widespread epidemic. Furthermore, since social jet lag has been previously associated with metabolic syndrome and obesity in an animal model, and since rats in the current study displayed free-running behaviour similar to animals experiencing models of shift work, it is evident that both this area of research, and the current animal model of social jet lag, have great potential to provide beneficial findings.

Additionally, the current study has provided novel insights into the FEO, such that advancements can be made in this research area to further investigate the relationship between the hippocampus and the FEO. Given that the hippocampus is typically the first brain structure to become affected by Alzheimer's disease, and subsequently the most heavily damaged (West, Coleman, Flood, & Troncoso, 1994), understanding how FEO access can improve hippocampal-dependent memory and learning can be monumental to Alzheimer's patients. However, in addition to benefitting individuals with neurodegenerative diseases, advancing the understanding of methods to improve learning and memory, in general, is beneficial for all individuals.

In conclusion, it is evident that circadian rhythm disruption is an area of research in need of further investigation. With technological advancements on the rise, along with increased societal demands, circadian rhythm disruption is becoming commonplace. Utilizing animal models to understand the intricacies of endogenous circadian oscillators, in addition to their impact on learning and memory, is undoubtedly imperative to protect

individuals against the wrath of diseases and disorders associated with the circadian rhythm disruption consuming our population.

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Table 1

Social Jet Lag Manipulation (SJM) Schedule

Day	Lights On	Lights Off
Saturday	1100	0300
Sunday	1200	2400
Monday	0700	2300
Tuesday	0900	2300
Wednesday	0900	2400
Thursday	0700	2200
Friday	0800	0100

Figure 1. Experiment One timeline.

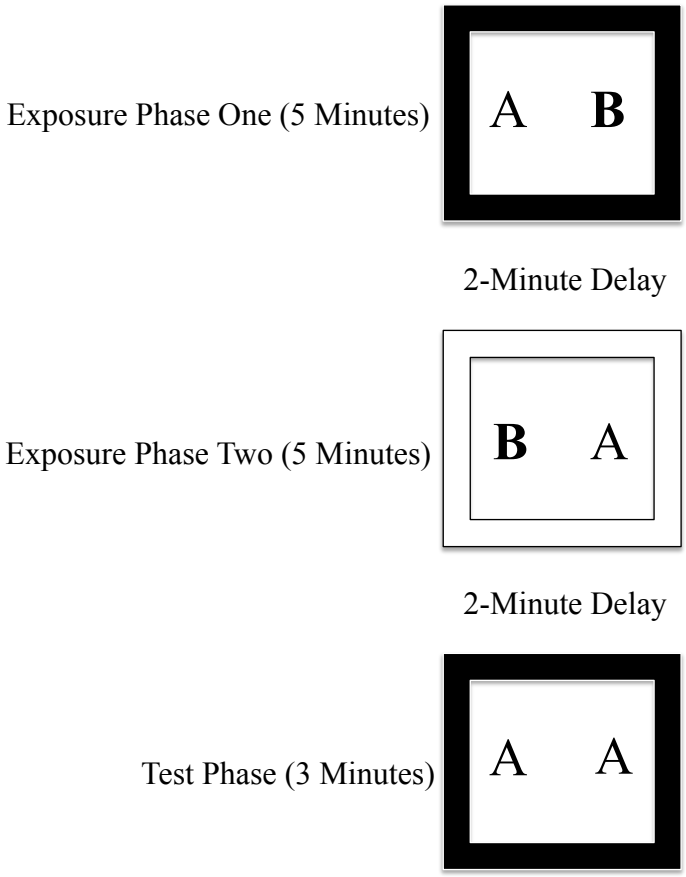

Figure 2. CDNOR procedure.

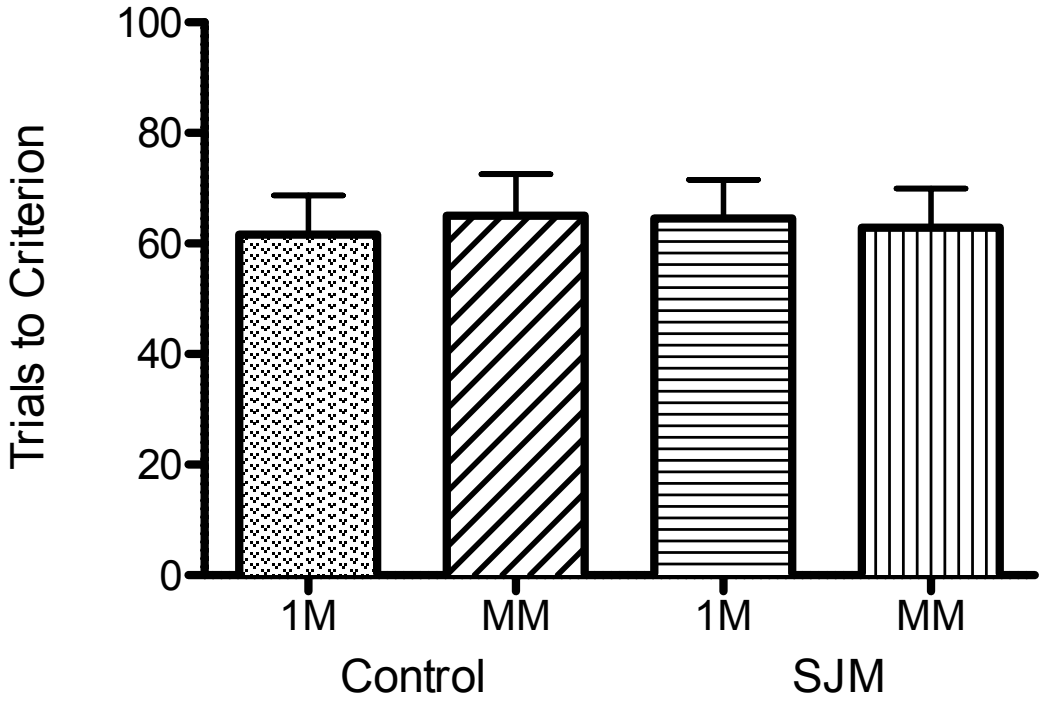

Figure 3. Average (±SEM) trials to criterion (18/20) on the SR task in Experiment One.

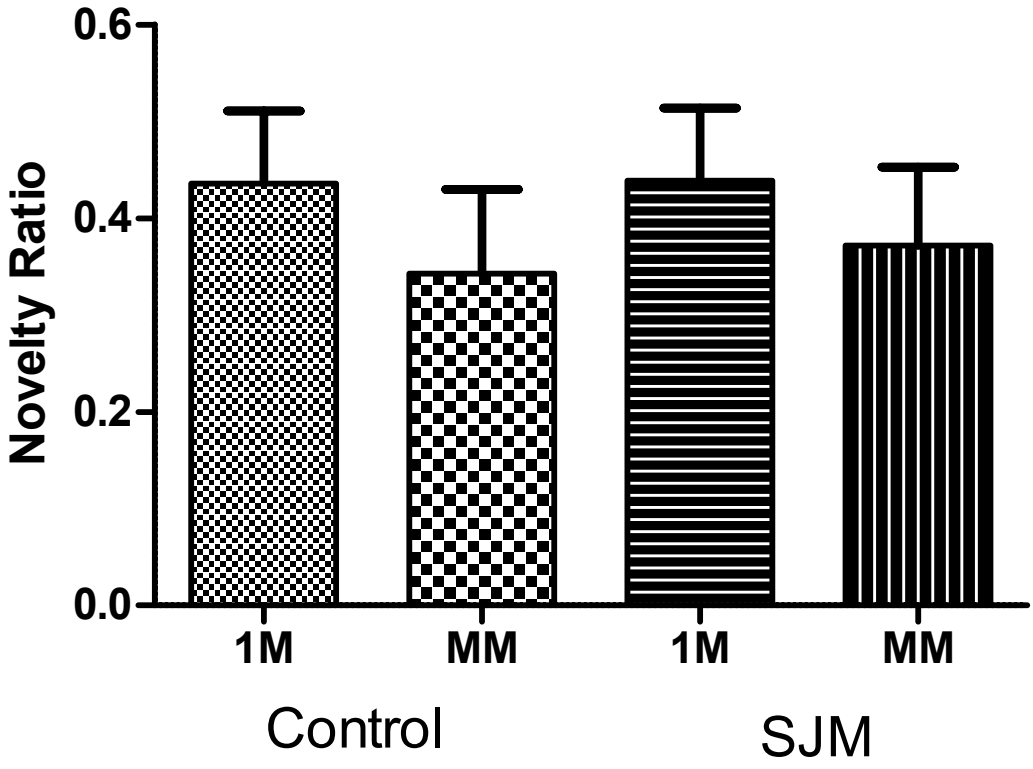

Figure 4. Average novelty ratio (±SEM) per group on the CDNOR task in Experiment One.

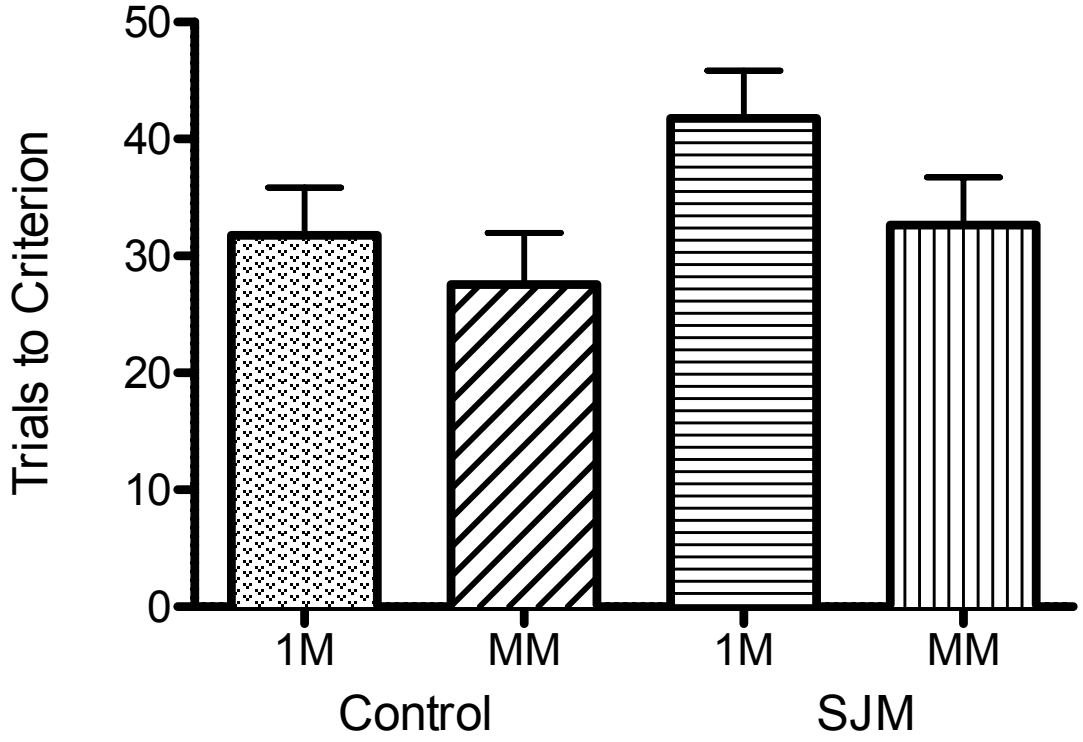

Figure 5. Average (±SEM) trials to criterion (18/20) per group, for the WPM in Experiment One.

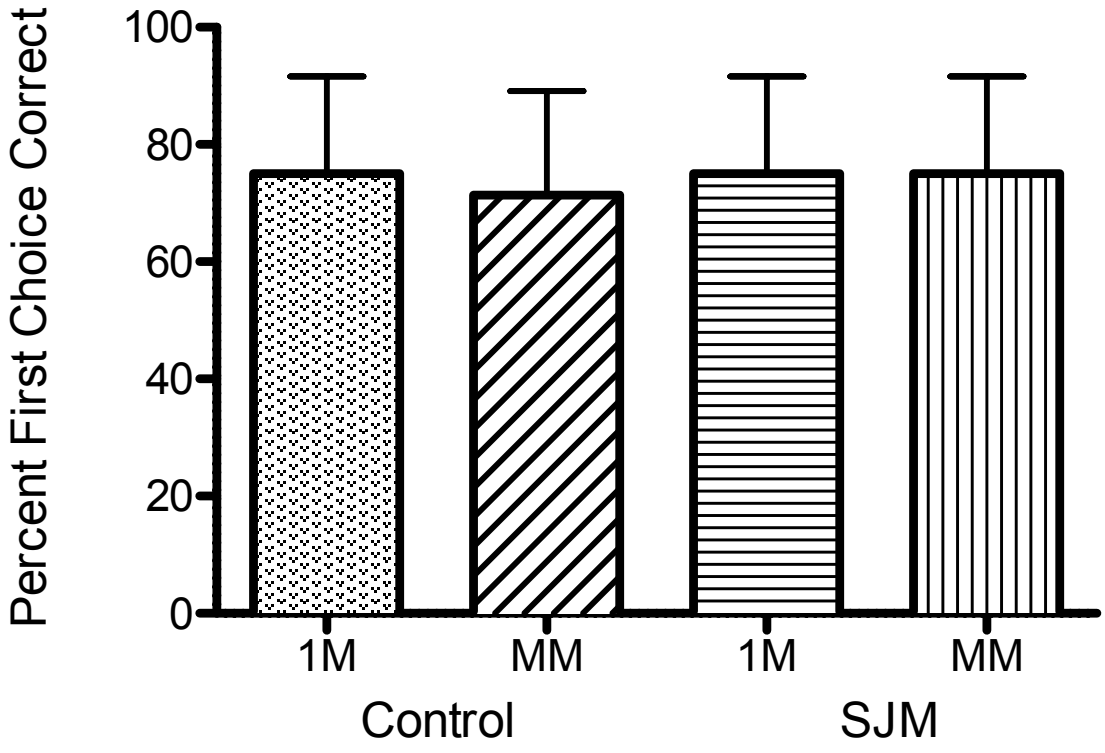

Figure 6. Percent first arm choice correct (±SEM) per group, during the WPM probe in Experiment One.

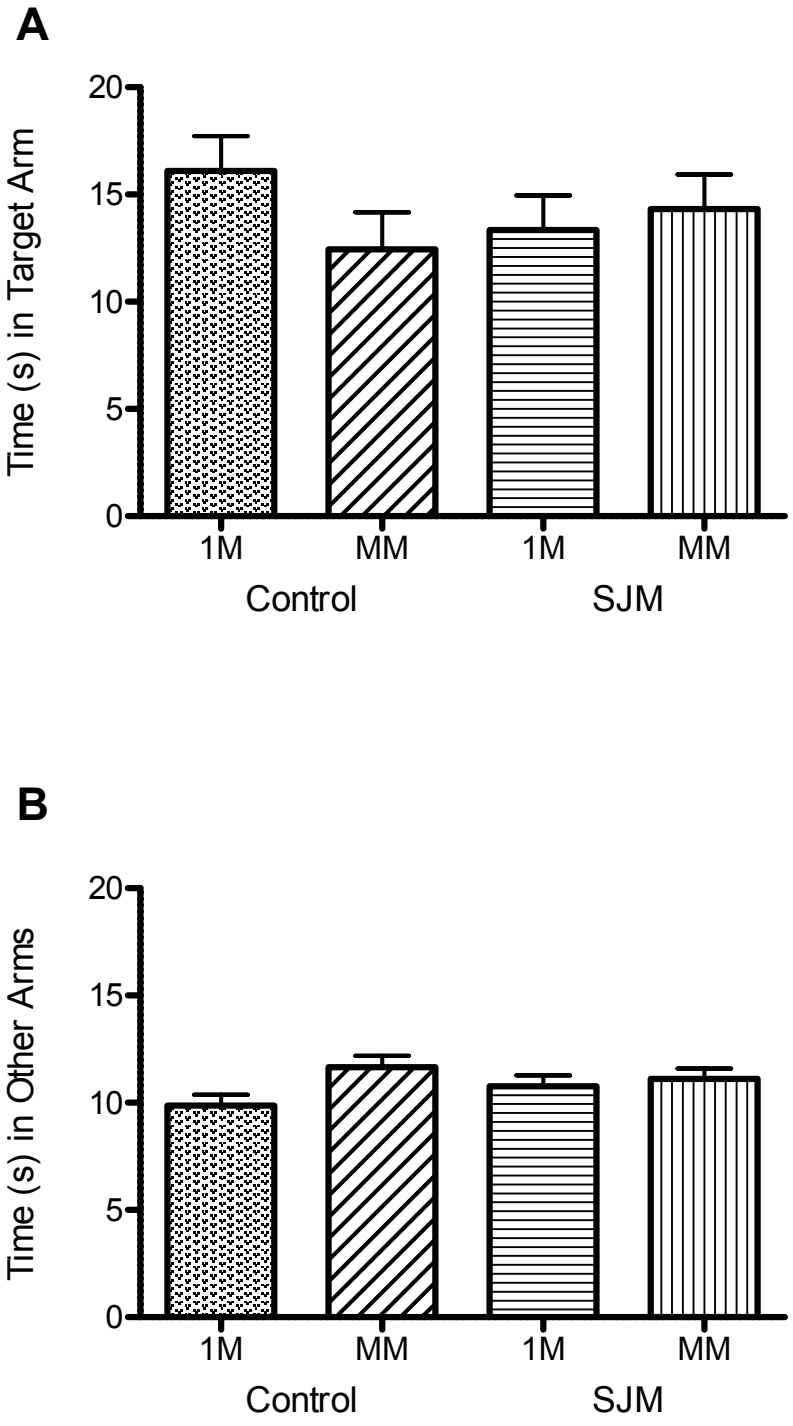

Figure 7. Average (±SEM) time in the target (A) and other (B) arms per group, during the WPM probe in Experiment One.

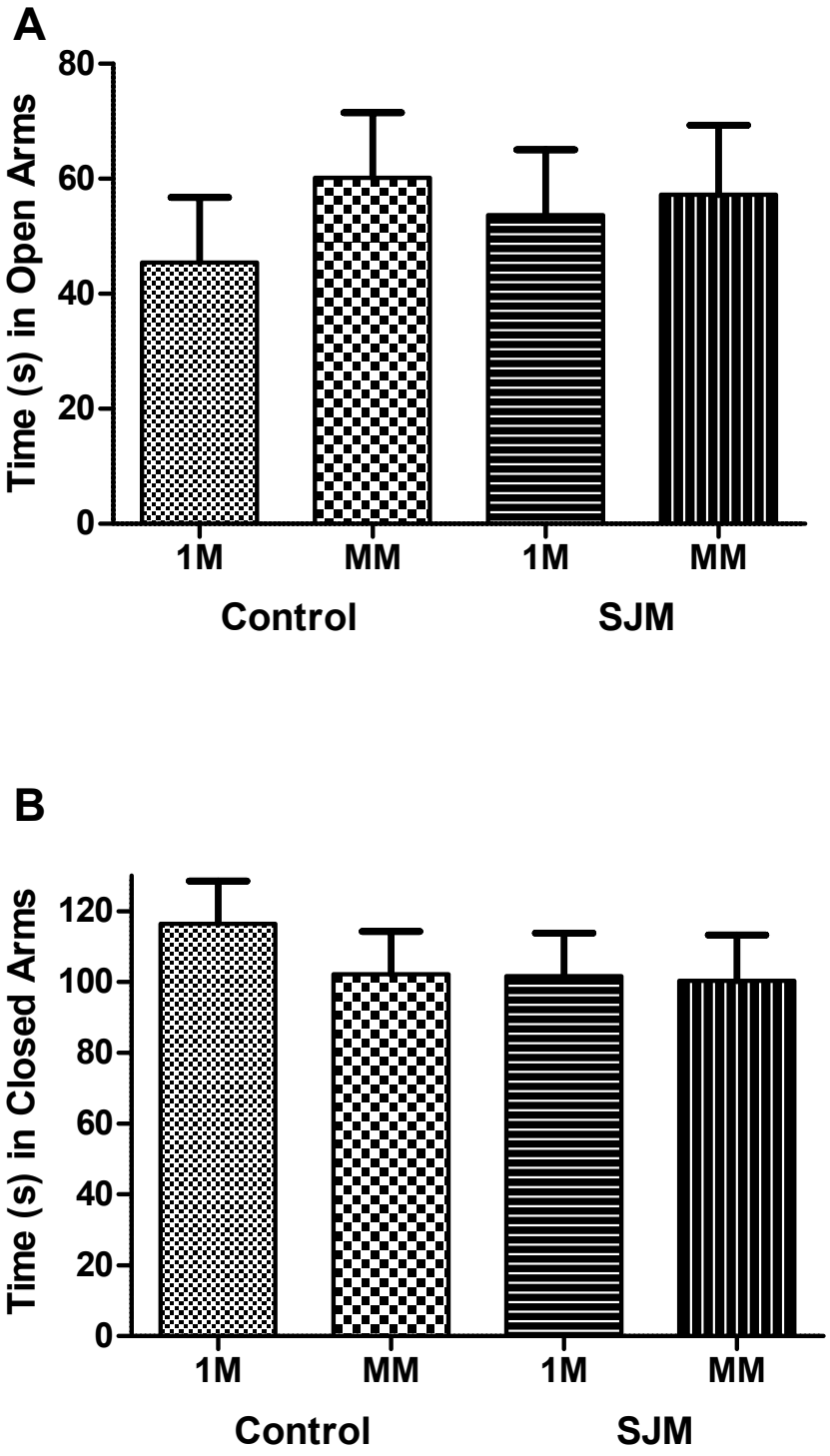

Figure 8. Average (±SEM) time in open (A) and closed (B) arms per group on the EPM in Experiment One.

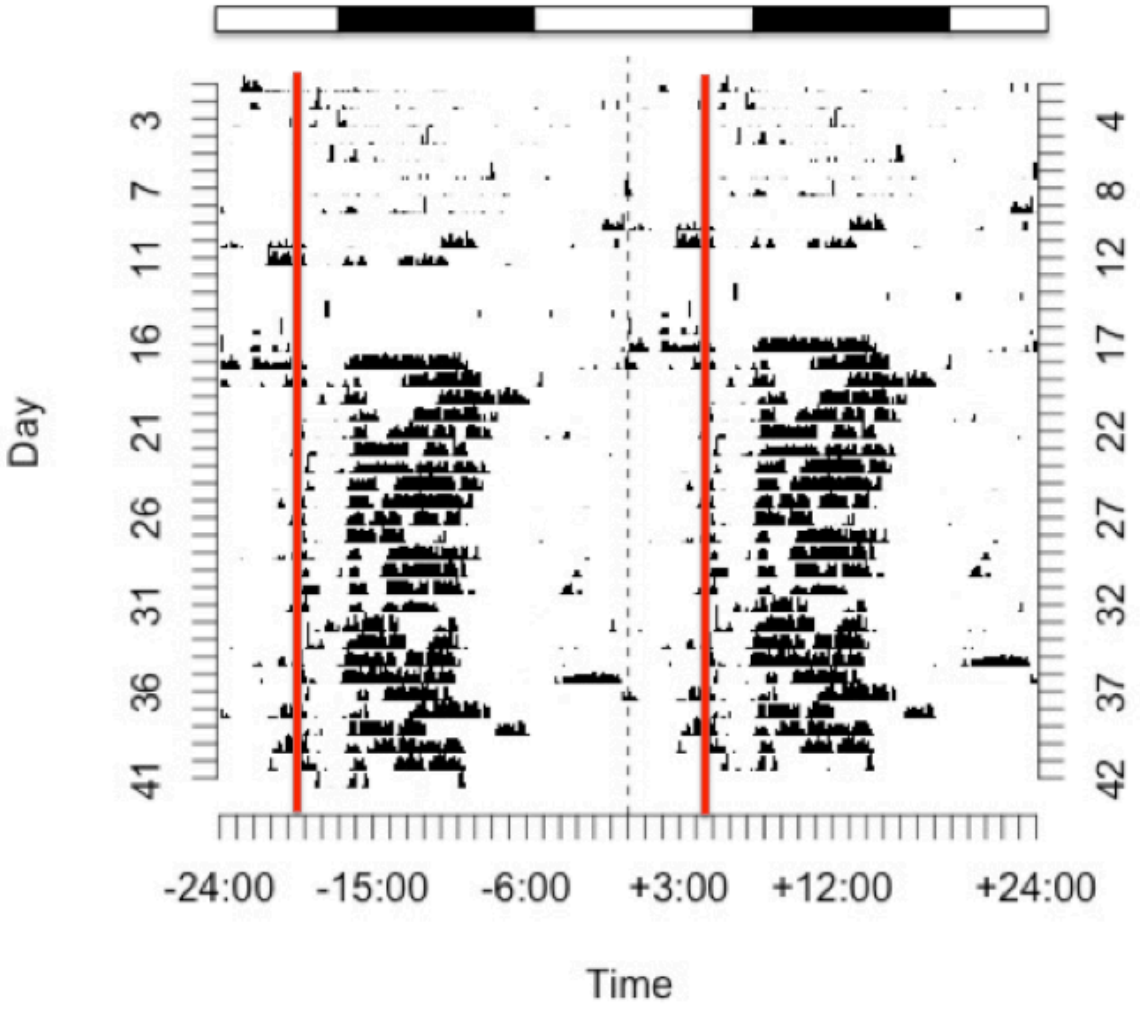

Figure 9. Actogram from the 1M-C group. This actogram depicts rhythmic activity of a rat exposed to a 12:12 LD cycle (red line represents mealtime; white and black bars represent lights on and lights off, respectively). Rats tended to be removed from wheels for training purposes between 900 and 1600 daily.

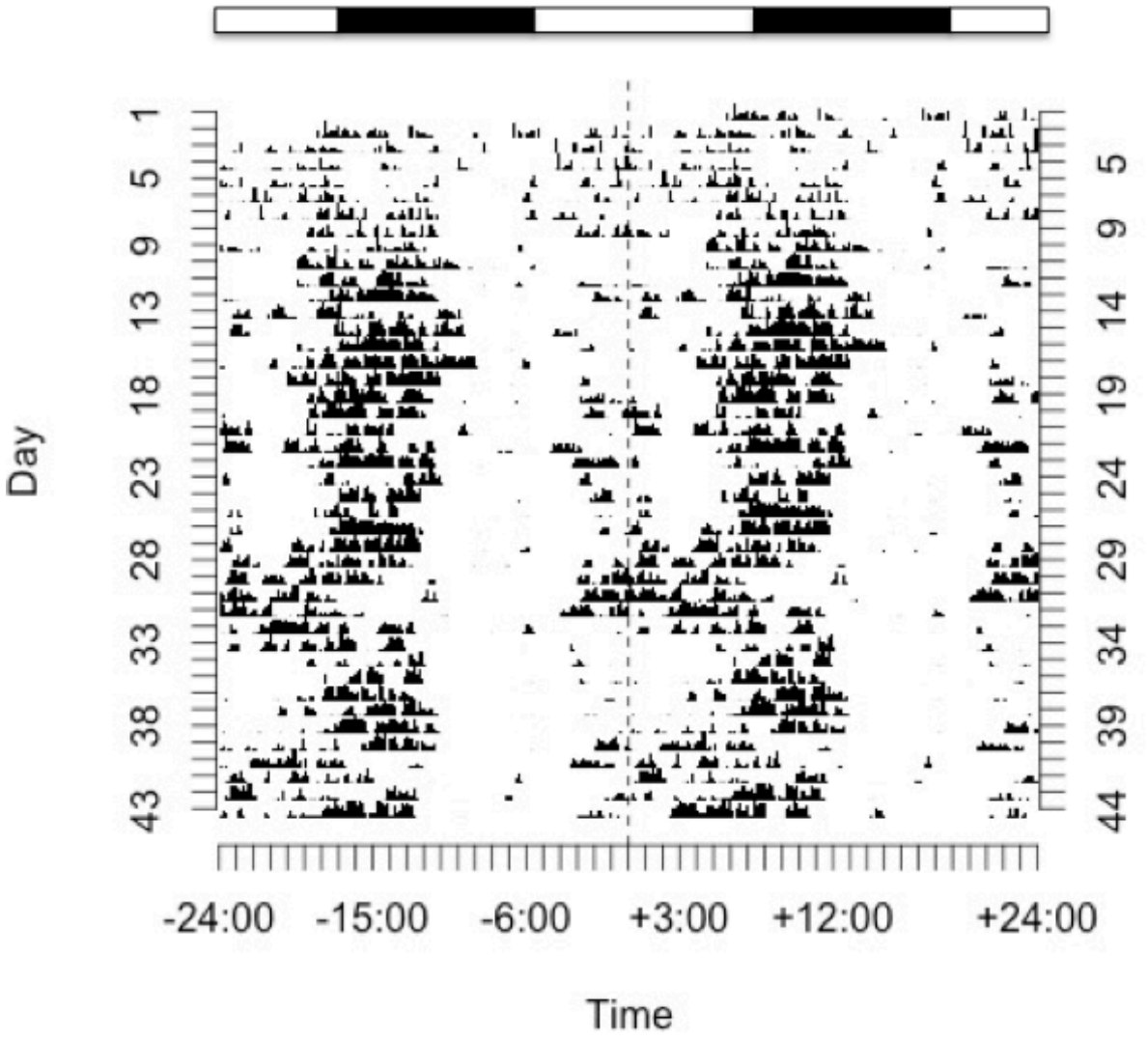

Figure 10. Actogram from the MM-C group. This actogram depicts rhythmic activity of a rat exposed to a 12:12 LD cycle (white and black bars represent lights on and lights off, respectively). Rats tended to be removed from wheels for training purposes between 900 and 1600 daily.

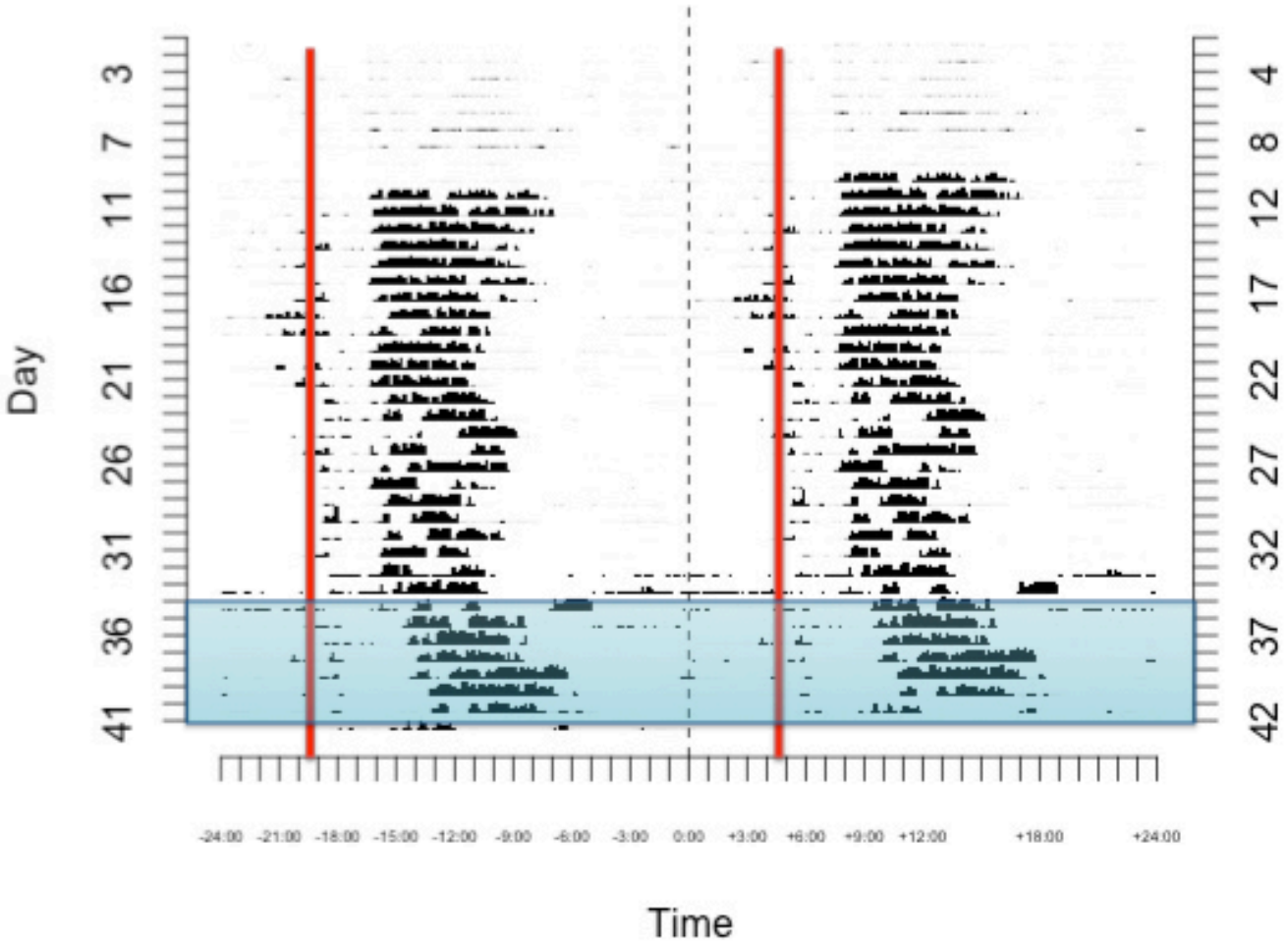

Figure 11. Actogram from the 1M-SJM group. This actogram depicts free-running activity of a rat exposed to the SJM emulating social jet lag (red line represents mealtime; lighting manipulation occurred during the transparent blue rectangle). Rats tended to be removed from wheels for training purposes between 900 and 1600 daily.

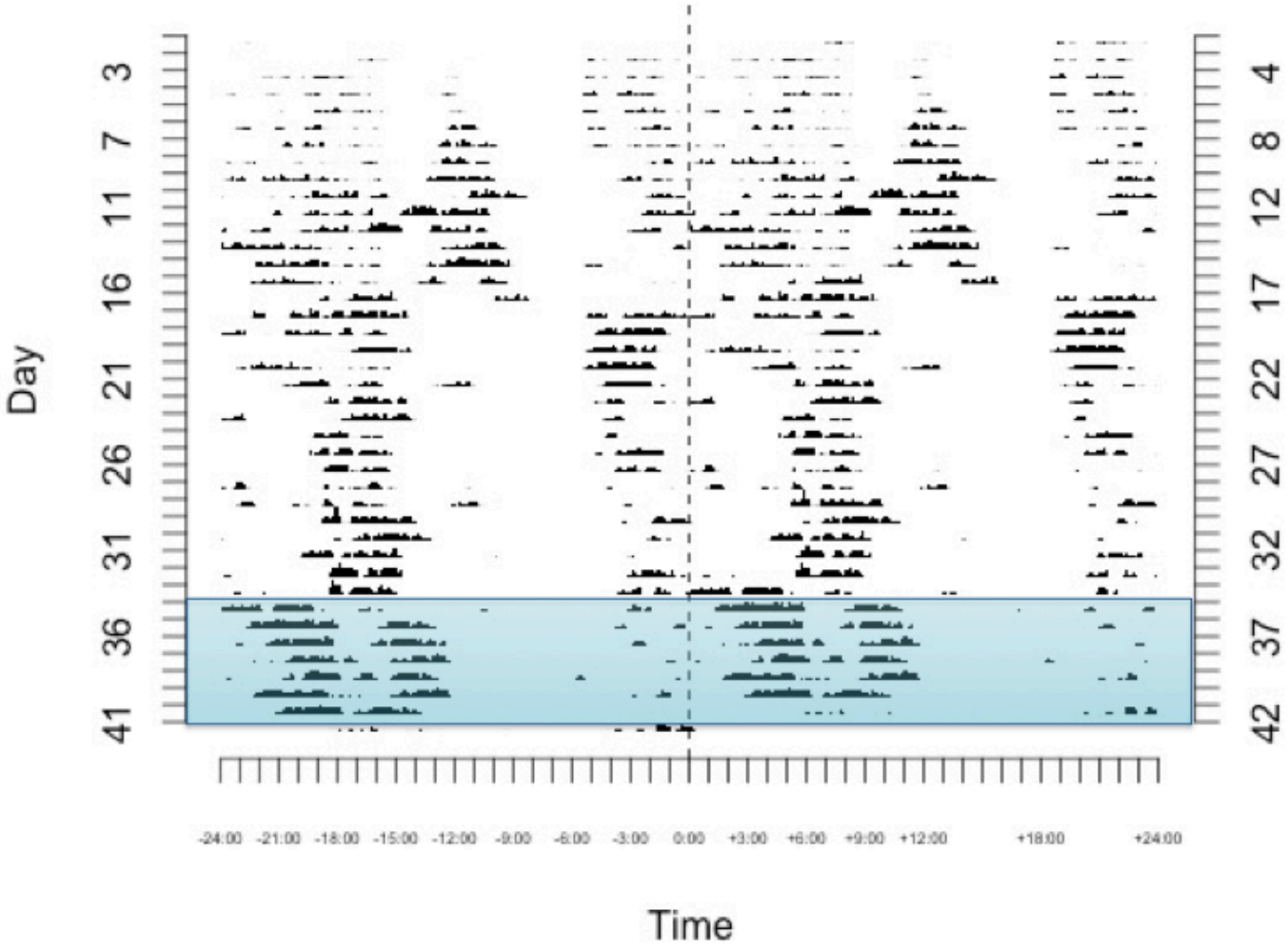

Figure 12. Actogram from the MM-SJM group. This actogram depicts free-running activity of a rat exposed to the SJM emulating social jet lag (lighting manipulation occurred during the transparent blue rectangle). Rats tended to be removed from wheels for training purposes between 900 and 1600 daily.

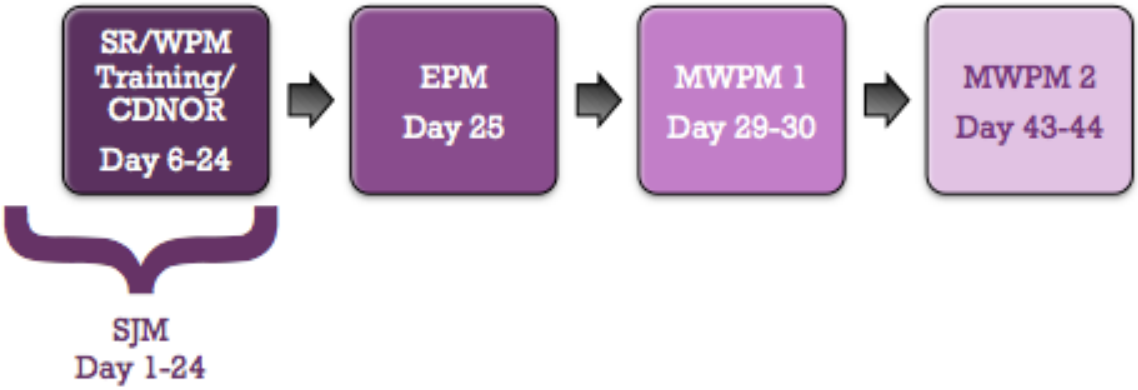

Figure 13. Experiment two timeline.

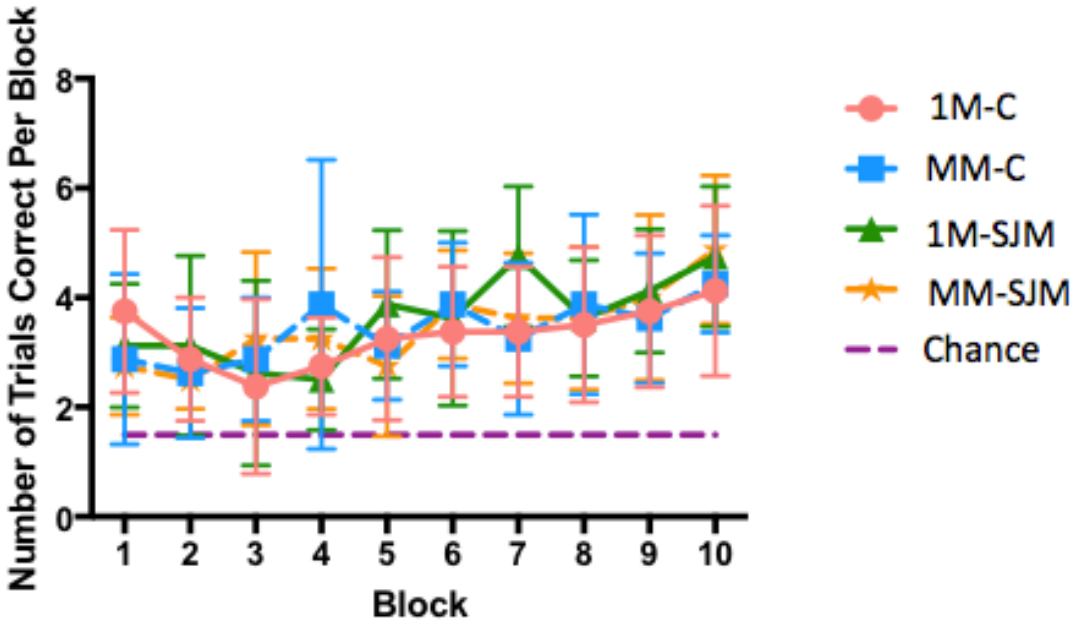

Figure 14. SR acquisition (±SEM) over 10 blocks of 6 trials, per group in Experiment Two.

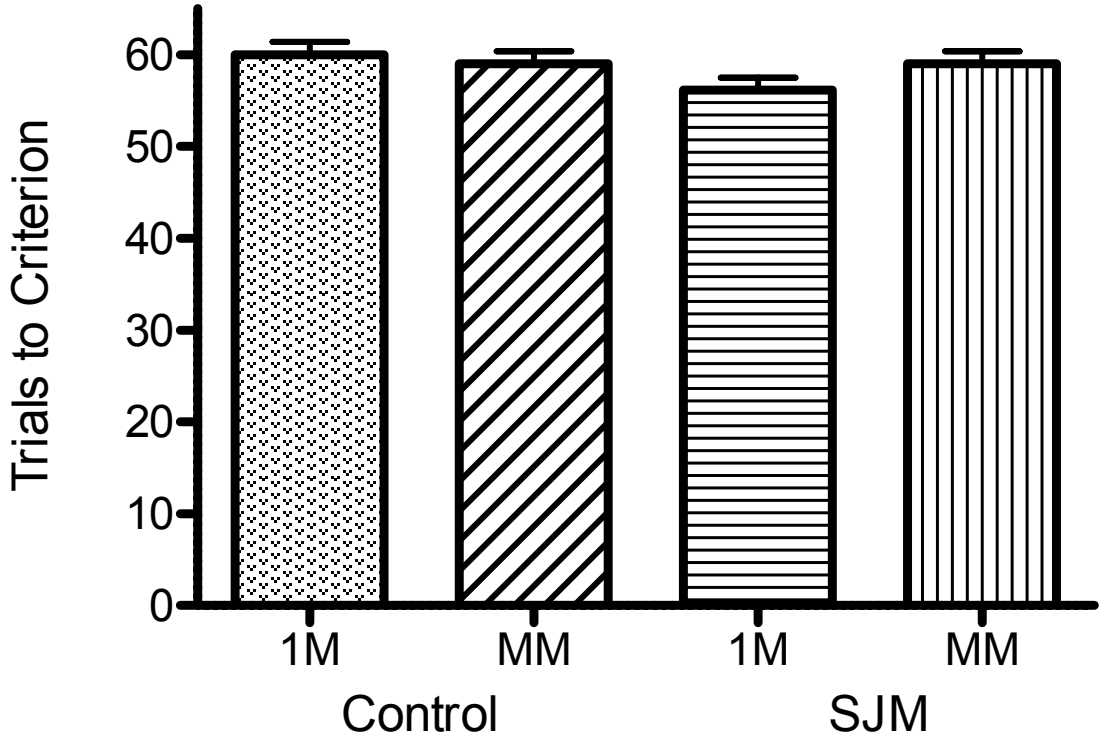

Figure 15. Average (±SEM) SR trials to criterion (18/20) per group in Experiment Two.

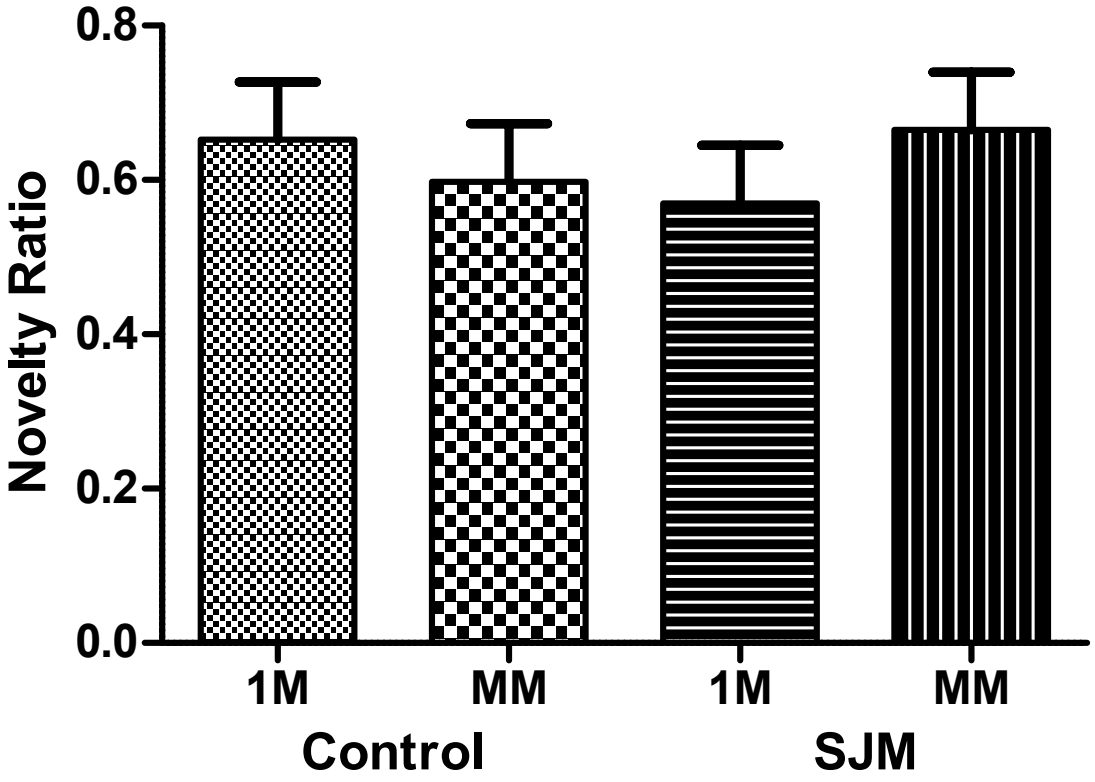

Figure 16. Average novelty ratio (±SEM) per group, on the CDNOR task in Experiment Two.

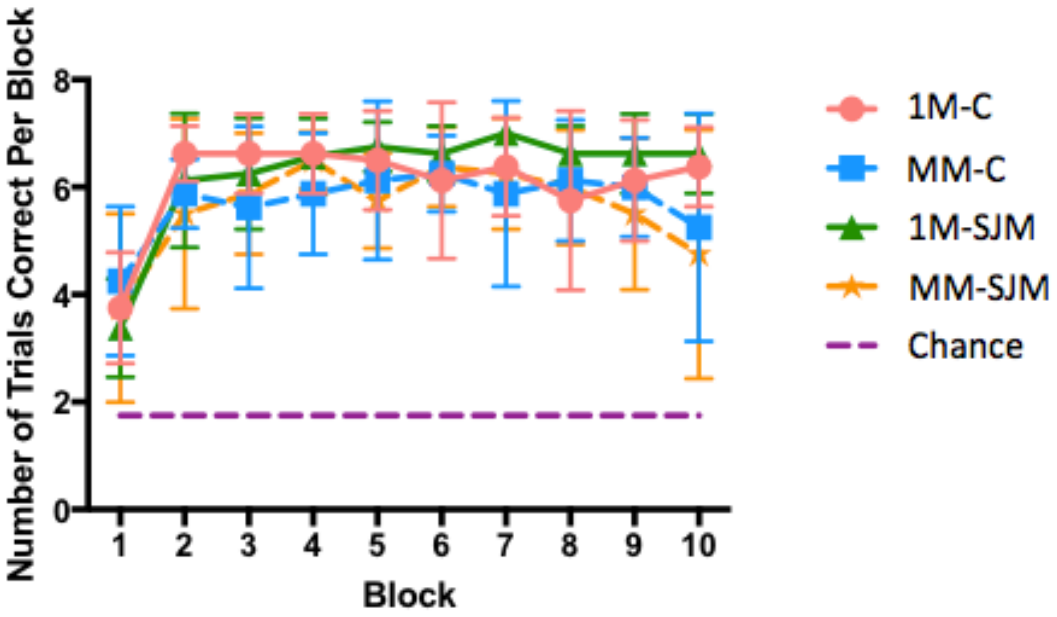

Figure 17. WPM acquisition (±SEM) over 10 blocks of 7 trials per group in Experiment Two.

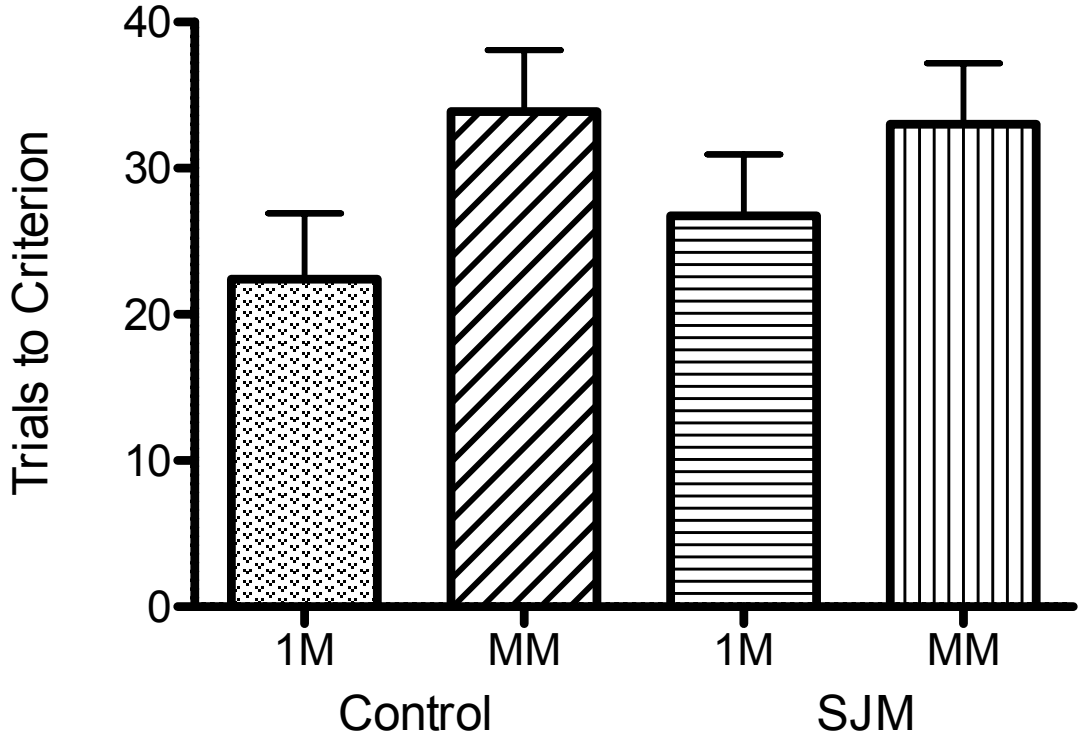

Figure 18. Average (±SEM) WPM trials to criterion (18/20) per group in Experiment Two.

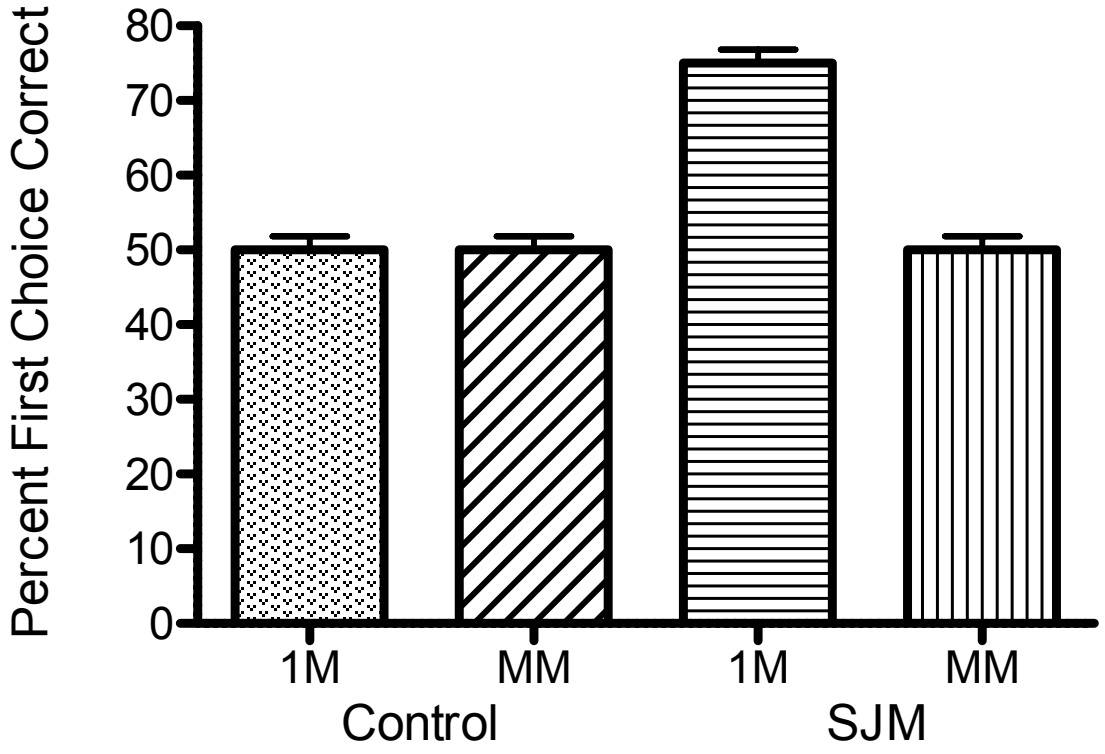

Figure 19. Average (±SEM) percent first arm choice correct during MWPM probe session one per group in Experiment Two.

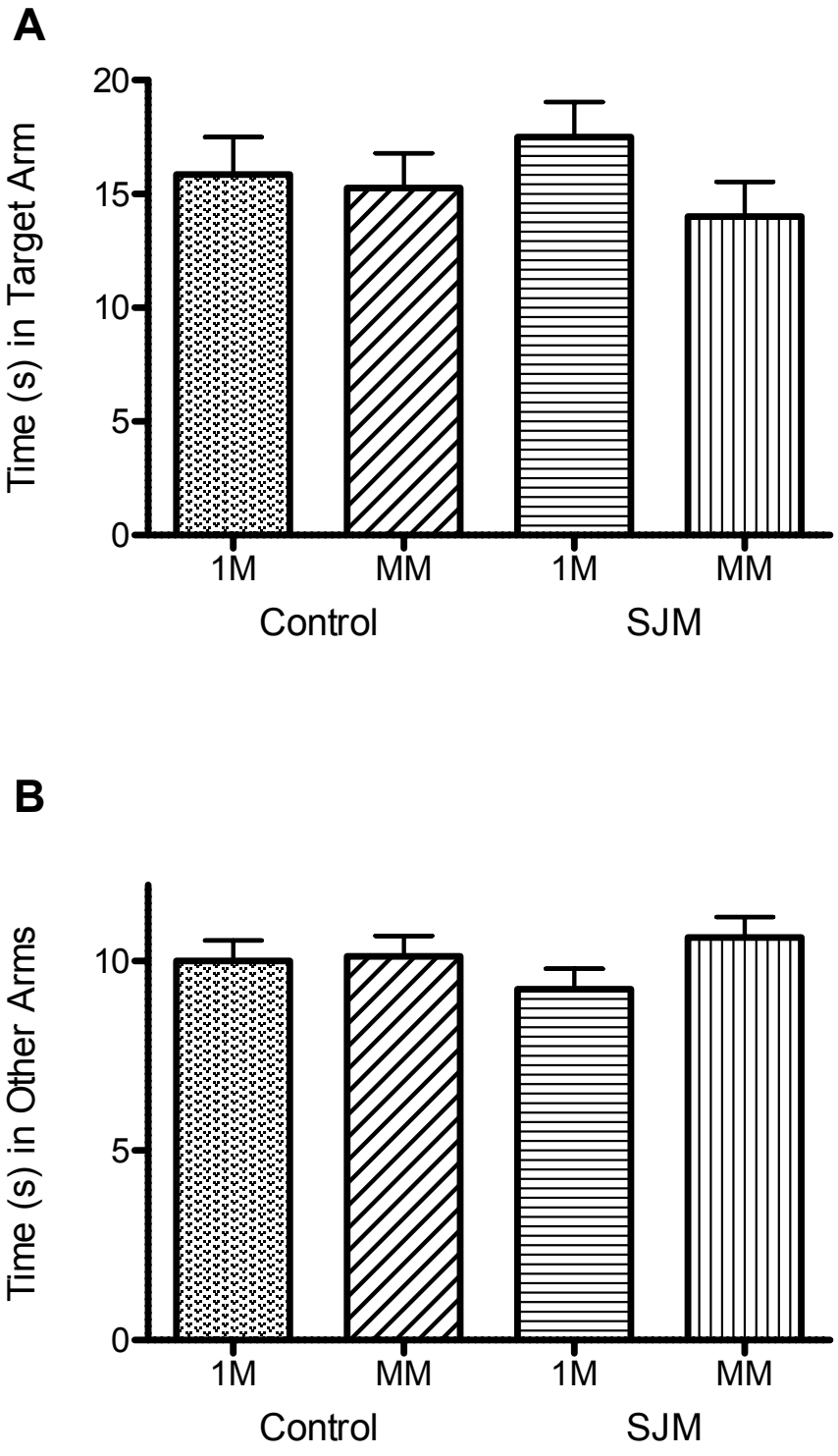

Figure 20. Average (±SEM) time in target (A) and other (B) arms during MWPM probe session one per group in Experiment Two.

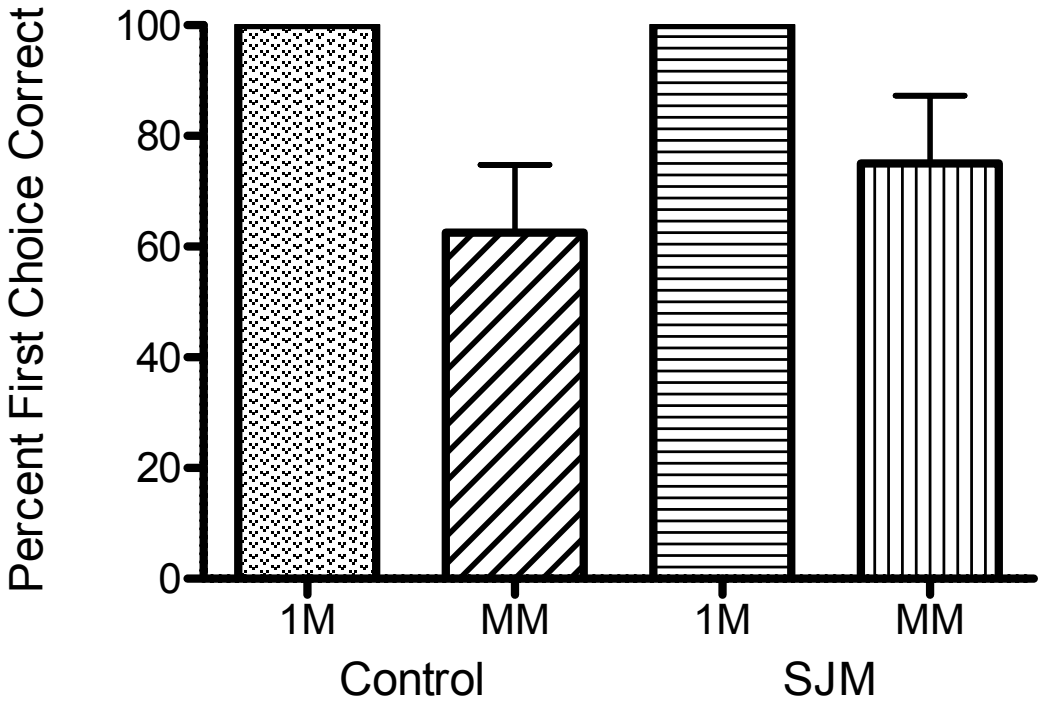

Figure 21. Average (±SEM) percent first arm choice correct during MWPM probe session two per group in Experiment Two.

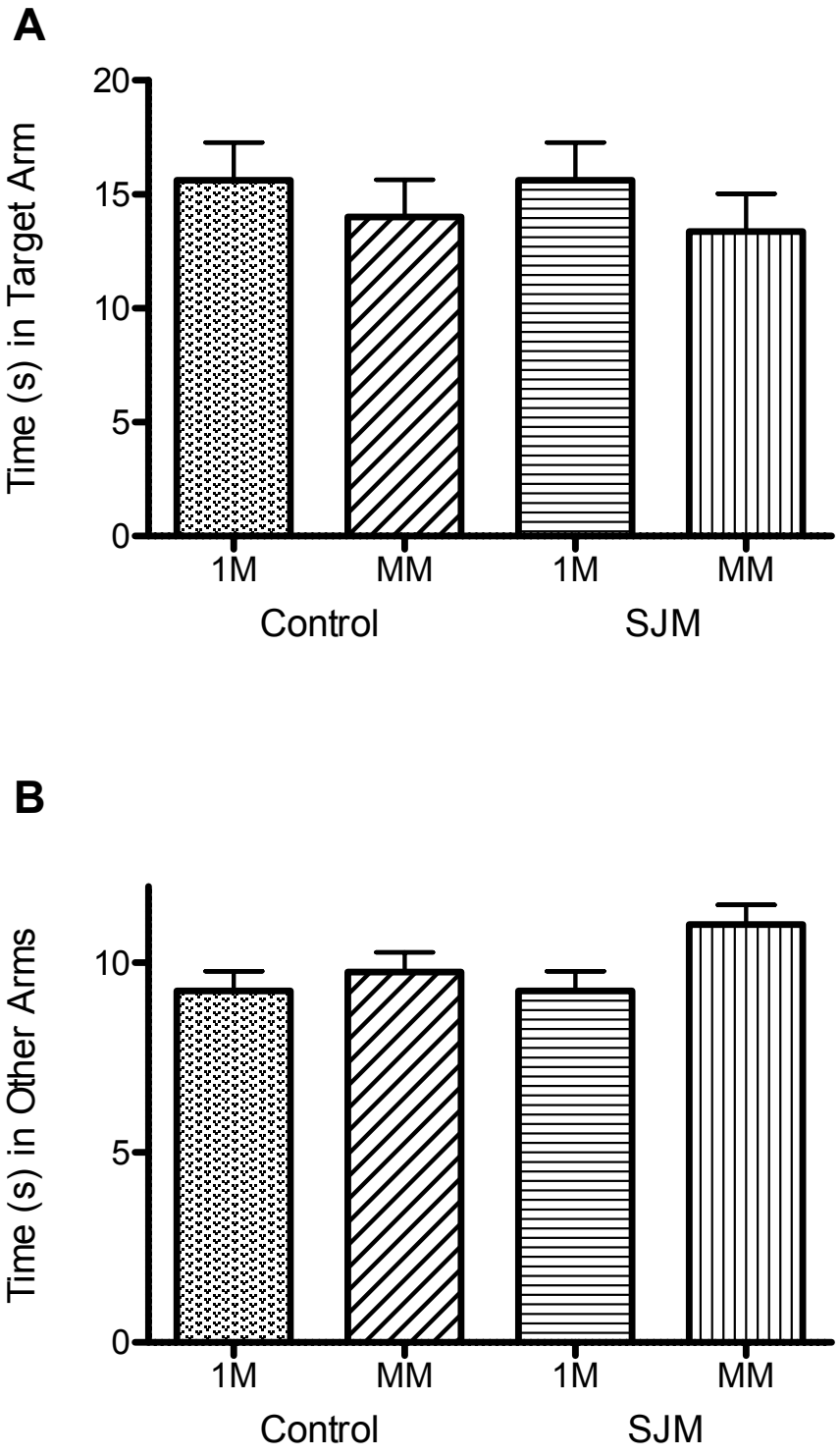

Figure 22. Average (±SEM) time in target (A) and other (B) arms during MWPM probe session two per group in Experiment Two.

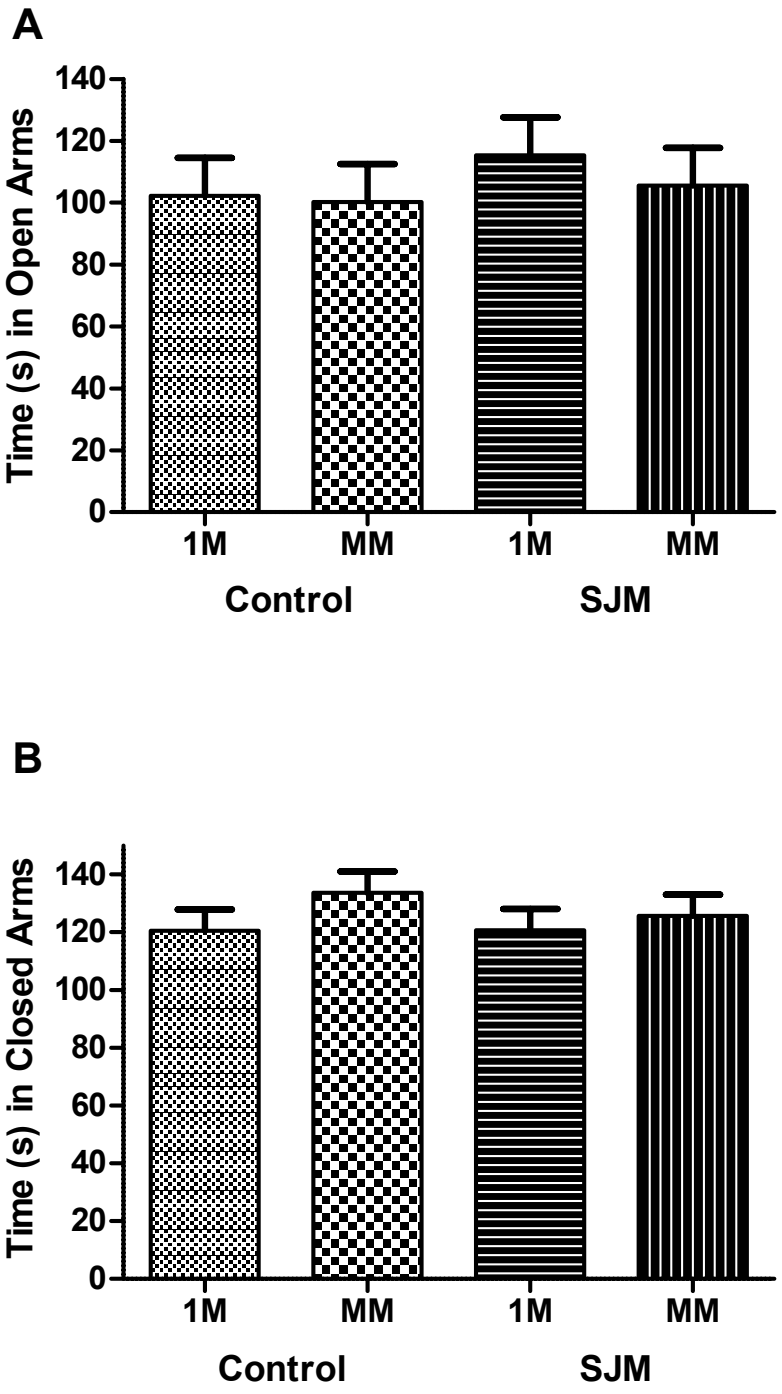

Figure 23. Average (±SEM) time in open (A) and closed (B) arms during EPM per group in Experiment Two.

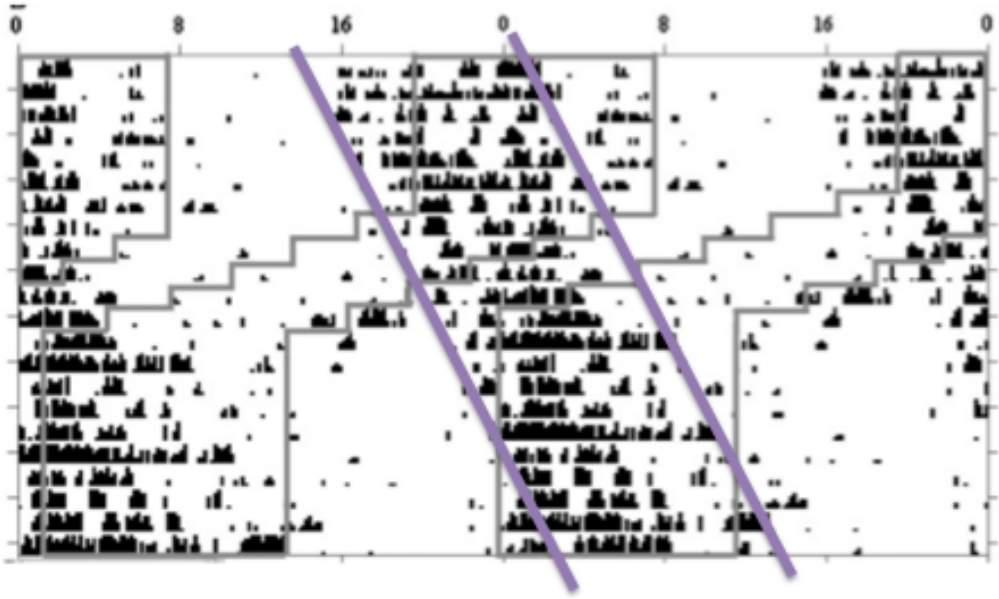

Figure 24. Modified actogram from Zelinksi et al. (2014). This actogram depicts free-running activity (as indicated by purple lines) of a rat exposed to a photoperiod shifting paradigm emulating shift work.