How do Changes in Meal Predictability and Timing Affect Hippocampal Dependent Tasks?

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Abstract

Light is the most prominent zeitgeber for circadian rhythms, but other stimuli such as food are also robust entrainers. Manipulations of the Light-Dark cycle impair hippocampal-dependent memory in rodents; however, little is known about the effects of meal timing on this type of memory. The purpose of these experiments was to determine the effect of manipulating meal timing and predictability on the Morris Water Maze (MWM) task. Rats received their food either in one meal at the same time of day (1M), multiple meals at random and unpredictable times (MM), or of their own choosing (*ad libitum*). Rats were trained on the MWM to find a hidden platform before their memory retention was tested using no-platform probes. In Study 1 (i.e., Standard Lighting), it was predicted that the 1M group would show better memory retention than the MM group. The 1M performed as expected by showing high memory retention; however, the MM group did not show a memory deficit likely due to issues with nocturnality. Thus, Study 2 was a replication of Study 1 that utilized a reverse lighting schedule such that meals and testing were administered during the dark cycle. The 1M and MM group continued to perform above chance, indicating that light was a robust entrainment tool that masked any potential effects of meal manipulation. Finally, Study 3 implemented a constant darkness manipulation to eliminate the influence of light. It was expected that rats with a disrupted lighting and feeding schedule (i.e., the DD-MM group) would show a memory deficit on the retention probes. This was confirmed, thus indicating that there is an additive effect of light and food as zeitgebers on hippocampal-dependent memory. Taken together, these studies provide evidence that light is the most prominent entrainment tool and, when available, the influence of lighting information will mask that of other zeitgebers. Studies of this nature will help elucidate the impact of disrupting zeitgebers not controlled by the suprachiasmatic nucleus.

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Biological rhythms are natural cycles generated from within the body that keep the internal time of many functions (Kleitman, 1949; Rusak & Zucker, 1975). Arguably the most prominent biological rhythm is the circadian rhythm (CR), which repeats an approximately 24-hour cycle and is governed by the suprachiasmatic nucleus, or SCN (Gritton, 2011; Zee et al., 2013). Many endogenous processes rely on our circadian rhythm, including the release of hormones throughout our body, our internal temperature changes, and our sleep-wake cycle (Czeisler & Klerman, 1998; Dibner et al., 2010). While our circadian rhythm is influenced by a variety of peripheral oscillators, the "master clock" is found in the suprachiasmatic nucleus of the hypothalamus. This area, comprised of two nuclei (i.e., the core and the shell) and over 20 thousand neurons, is responsible for the majority of circadian timekeeping (Silver & Kriegsfeld, 2014; Zelinski et al., 2014). Although our circadian rhythm is controlled by both primary and peripheral oscillators, they require the input of zeitgebers to keep accurate circadian timing. German for "time-keepers or time-givers", zeitgebers act as external cues to synchronize the body with the natural environment (Gritton, 2011). Examples of such cues include light, food intake, temperature, and social influences (Dibner et al., 2010; Zee et al., 2013).

Light is the most robust entrainment tool, with the most obvious example being the natural rising and falling of the sun. However, artificial light such as that from lightbulbs and electronics can also provide entrainment (Honma, 2018; Vitaterna et al., 2001). Light information is registered by the eyes via photoreceptors of the retina. From here, this light information is sent along the retinohypothalamic tract to the SCN. Once the information is received in the SCN, it relays time-of-day information to the rest of our body, essentially guiding

it to behave a certain way at a particular time (Honma, 2018; Vitaterna et al., 2001). This mechanism by which light entrains our circadian rhythm is termed the light entrainable oscillator (LEO). This mechanism is crucial survival, as time-of-day information is necessary for maintaining feeding, mating, and sleeping behaviors in non-human animals.

While light remains the most influential zeitgeber due to its large effect on the SCN, food intake works on a semi-autonomous oscillator termed the food entrainable oscillator (FEO) to maintain a proper circadian cycle in the body (Mistlberger, 2011). Essentially, organisms can anticipate when they will have access to food if that access is granted in a small number of consistent times throughout the day, with one meal per day allowing the most predictability (Lukoyanov et al., 2002; Wall et al., 2019). Evolutionarily, this would have served as a survival tool as it is advantageous to be able to predict when the next meal will occur in order to conserve energy and maintain metabolism. The observable product of the FEO is food anticipatory activity (FAA), which is simply the increase in locomotor activity starting a few hours before feeding, if the mealtime(s) are consistent each day (Mistlberger, 2009; 2011). If the feeding times are not consistent, then FAA is typically not present, and the FEO is not activated. Given that FAA remains during constant conditions such as fasting, and it follows a circadian timeline of approximately 24 hours, this mechanism was termed FEO to represent the circadian nature (Mistlberger 2009; 2011). However, the FEO does not share the same location in the brain as other time-keeping mechanisms. Lesion studies, where the SCN and hypothalamus, hippocampus, amygdala, and other brain regions were compromised found FAA remained intact (Mistlberger & Mumby, 1992; Mistlberger & Rusak, 1988). Although mutant and knock-out rodent models have been shown to alter the period or expression of FAA via gene alteration (Pendergast & Yamazaki, 2018), this simply tells us which genes may be involved, and it does

not identify the location of the FEO. Due to the elusive nature of this mechanism, more research is needed to further understand the anatomical basis of the FEO.

Disruptions to CR have been shown to influence behavior and cognition in addition to physiology (Chaudhury & Colwell, 2002; Gritton et al., 2012; Gritton et al., 2013). A circadian rhythm disruption (CRD) can occur in two ways. First, a misalignment of our CR timing and the external environment can create a desynchronization. Typically, this occurs by having nighttime light, morning darkness, or irregular and opposite eating habits (Czeisler & Klerman, 1998; Karatsoreos et al., 2011). Second, a dysfunction of the central and/or peripheral clocks and their respective communication pathways can produce a CRD (Karatsoreos et al., 2011; Zee et al., 2013). Examples of such dysfunctions are a delayed or advanced sleep phase or sleep-wake disorders. As well, this type of disruption is induced in animal research by lesioning the SCN or creating knockout models (Gall et al., 2016; Phan et al., 2011; Shimizu et al., 2016). These two versions of CRD are not contrasting—in fact, they can occur simultaneously to create an even more complex form of a disruption.

When such CRD are present, adverse effects on sleeping, eating, metabolism, and cognition may occur. As well, CRD has been associated with mood disorders, cancer, and Alzheimer's disease (Zelinski et al., 2014; Zelinski et al., 2013). The influence of CRD on cognition, namely memory, is the focus of the current study. Specifically, past research has shown that the hippocampus is particularly vulnerable to CRD (Devan et al., 2001; Loh et al., 2010; Phan et al., 2011; Ruby et al., 2008). Spatial learning and memory tasks are often utilized in this area of research, as this type of task is mediated by the hippocampus. Animals are put through an acquisition (i.e., learning) phase before completing retention testing. For example, rats subjected to a six-day, three-hour photoperiod shift had significantly impaired platform

location retention in the Morris Water Maze task (MWM), a task specific to the hippocampal region of the brain (Devan et al., 2001).

Inactivation or disruption of the FEO has similar effects to disruptions of the sleep-wake cycle on learning and memory in rodents (Lukoyanov et al., 2002; Mistlberger et al., 1996; Wall et al., 2019). For example, Wall et al. (2019) manipulated the FEO by feeding rats at either consistent (1M per day, 6pm) or unpredictable (MM per day, between 10am and 7pm) mealtimes. Those fed 1M per day presumably had access to the FEO, while those fed multiple, unpredictable meals per day would not have had access to the FEO. They were then trained on a daily Time-Place Learning (TPL) task in which the location of a food reward varied depending on the time of day. The rats fed 1M outperformed those fed at unpredictable times. The purpose of this thesis is to determine whether access to the FEO improves memory and whether disrupting the FEO with unpredictable mealtimes impairs memory. To do this, we compared 1M, MM, and *ad libitum* groups in a hippocampal-dependent water maze task under different lighting conditions.

Study 1: Standard Lighting

Rats fed one meal at a consistent time each day, performed better on a daily TPL task compared to rats that were fed multiple meals per day at unpredictable times (Wall et al., 2019). However, it is possible that consistent mealtimes only provide an advantage in daily TPL tasks in which circadian information is needed to solve the task. The purpose of the first study was to determine the effect of consistent mealtimes on learning and memory. Specifically, we focused on the hippocampally-dependent MWM. The photoperiod shifts induced by the McDonald lab (i.e., three-hour phase advances for six consecutive days) resulted in impairment in both acquisition (Zelinski et al., 2014) and retention (Devan et al., 2001) in the MWM.

We also expanded on the Wall et al. (2019) study by including activity monitoring and the addition of an *ad libitum* (AD) group. Wall et al. (2019) found that rats fed one meal at the same time each day outperformed rats fed multiple meals per day at unpredictable times. However, without activity monitoring we do not have a clear understanding of the impacts of these manipulations on CRs. Also, the addition of an AD group will allow us to determine if one meal (and access to the FEO) results in improved performance, or if multiple, unpredictable meals (and perhaps disrupted CR) results in impaired performance.

Therefore, in Study 1 we randomly assigned rats to one of three groups: 1M (received one meal per day at 10 am and presumably would show FAA), MM (received multiple meals during the light phase at unpredictable times and presumably would not show FAA), and AD (*ad libitum*). Rats' activity was monitored throughout the experiment using either running wheels or activity sensors to detect FAA. We then looked at acquisition and retention in the MWM.

Based on the findings from Wall et al. (2019), it was hypothesized that 1M rats would outperform the MM rats as shown by improved acquisition and retention. However, how these groups will compare to the AD group is unknown. With respect to FAA, we expect to see evidence of FAA in the 1M group, but not in either the MM or AD groups.

Method

Animals and Housing

Thirty-two male Long Evans rats from Charles River Laboratories (QC, Canada) arrived weighing approximately 250 g. Only male rats were used for these studies as the female estrous cycle (i.e., hormonal variation and/or changes) would add another variable to account for when studying circadian rhythms. Thus, the inclusion of female rats is planned for future projects. Following their arrival, all rats were singly housed in individually ventilated cages (IVCs) (32

cm x 35 cm x 18 cm) which included corncob bedding (Netco, New York, NY)**,** Crink-l'Nest (The Anderson, Maumee, Ohio), a Nylabone (Nylabone Products, Neptune, NL) or wooden block, and a piece of plastic pipe for enrichment. All cages were kept in a temperature-controlled room and kept on a 12:12 light-dark cycle, with a light phase from 7:00 to 19:00. All rats remained under these housing conditions for 10 days for habituation purposes. After this, 24 rats were transferred to individual cages (40 cm x 18 cm x 17 cm) connected to running wheels (36 cm in diameter) while the remaining rats resided in their original cages with motion sensors. Both the conventional and running wheel cages contained the same materials as described in their initial description. After 10 days of acclimation to the wheels, the food manipulation phase of the experiment began. During transportation for behavioral testing, all rats were transferred to IVCs lined with paper towel for water absorption.

Activity and Feeding Schedules

Twenty-four rats were housed in cages attached to wheels; thus, their activity was monitored through a homemade Python script which continuously counted the number of wheel rotations. Despite not being in wheels, the activity of the remaining eight rats were monitored via a sensor connected to ClockLab Analysis 6 (Actimetrics, Wilmette, IL, USA). Before the food manipulation began, all rats had *ad libitum* access to water and standard rat food (PMI Nutrition International, St. Louis, MO). They were then separated into three different feeding groups. The first group was kept under the same *ad libitum* conditions as described previously. The second group was fed once a day consistently at 10:00 am. The final group had food administered one to three times a day at unpredictable times during the light phase that were at least two-hours apart and between the hours of 9:00 and 18:00. There were 11 rats in the 1M group, 11 rats in the MM group, and 10 rats in the AD group. All rats were food deprived (~90% of *ad libitum* food

amount) to a level in which growth of approximately ten grams each week was permitted. The rats remained on these feeding regimes until the end of the experiment.

Morris Water Maze

The MWM was a circular pool on a metal frame (178 x 178 cm) raised 28 cm off the floor by wheels. The pool had a diameter of 172 cm and the wall of the pool was 60 cm high. The water was filled to approximately 10 cm below the top of the pool and the temperature was maintained at approximately 21 to 23 degrees Celsius throughout the experiment. To conceal the location of the platform, the water was made opaque by adding white, washable, non-toxic paint (Prang & Dixon Ticonderoga, Newmarket, ON). The platform was 11 cm in diameter and positioned so the top of the platform remained around 2 cm below the surface of the water. It was made of white tubing which also allowed for height adjustments and was weighed down by sand. The room in which the experiment was conducted (357 x 592 cm) contained a variety of visual cues such as shelving, a table, lamps, and posters that all remained consistent throughout each phase.

During testing, the rats were brought to the testing room in labelled IVC cages and were then transferred to individual labelled IVC cages lined with absorbent paper towels. The room was dimly lit to allow for accurate video recording and a radio was turned on to conceal any background noise coming from outside the training room. Rats were released from one of four potential release positions (1, 2, 3 or 4) during each trial. The order of the release positions was pseudorandomly determined prior to beginning the experiment so each rat was released from each position twice per session. The water was agitated, and fecal matter was scooped out of the pool in between each trial to eliminate possible odor trails.

MWM began at 11:00 each day for all phases of testing. Before starting each trial, the rat in the testing cage was walked clockwise to a wooden chair located at the release position. Each trial started with the rat being placed at the release position facing the wall of the pool. The experimenter remained at the release position until the end of each trial. The rat was then permitted to swim freely until it reached and climbed onto the platform, or until 60 seconds had elapsed. If the rat found the platform within the given time, it was allowed to stay there for approximately 5 seconds before being removed from the maze. If the rat did not find the platform within the 60 seconds, the experimenter guided them to the platform where they could stand and wait for approximately 5 seconds. The experimenter then removed the rat, placed them back into the cage, and walked counterclockwise back to the bench. Each trial was recorded using a camera mounted above the maze using Blackmagic Video Recorder.

When conducting the two probe phases of the experiment, all details listed above remained the same except the platform was removed from the maze. Each rat was given 60 seconds to swim freely about the maze before being removed from the maze by the experimenter.

To assess the ability of a rat to acquire and retain spatial memory, a MWM paradigm consisting of acquisition training, mass training to a new location, and probe tests was used. After food manipulation, the rats began the acquisition training phase of the experiment in the water maze. The acquisition training involved eight training trials per rat per day to a static platform location. Each rat had a maximum of 60 seconds to find and stand on the platform located in a specific quadrant of the water maze. If they did not locate the platform, they were guided towards the platform location. This was repeated over four days in which the platform remained unchanged. Immediately after this, there was a seven-day break before the first probe

phase of the experiment. During the probe, the rats received a 60 second no platform trial. The following day, the mass training phase commenced and consisted of 10 training trials to a new platform location which remained the same throughout all trials. This training only lasted one day. Each rat had 60 seconds to find and stand on the platform, identical to the acquisition phase. Finally, the next day the rats received another 60 second no platform probe trial.

Data Analysis

Videos of each trial were recorded during the water maze task and were analyzed using Ethovision XT (Noldus). Ethovision measured and analyzed the latency to reach the platform during acquisition and mass training. Ethovision recorded the amount of time spent in each quadrant, out of a total of 60 seconds in the pool, and created a percentage of total time for each quadrant. A python script was created to collect the number of rotations the rats made in their running wheels, counted per minute, and these numbers were analyzed in ClockLab Analysis 6 (Actimetrics, Wilmette, IL, USA). The sensor data were also collected and analyzed using ClockLab. Double plotted actograms were created such that dark periods in the rats' L:D cycle was shown as a shaded area on the graph.

Results

Morris Water Maze

Acquisition Training. The average latency to reach the hidden platform was analyzed across training day and meal group using a mixed model (ANOVA) with group as the between measures factor and day as the repeated measures factor. A significant effect of day was found, such that the rats took less time to reach the platform as the days progressed, $F(3,948) = 175.470$, $p < 0.001$, $\eta^2_p = 0.357$. There was not a significant effect of meal group, showing that all rats learned the location of the platform similarly, $F(2,948) = 1.626$, $p = 0.197$, Figure 1A. It must be

noted that two rats in the 1M condition were excluded from acquisition analyses, because performance exceeded two standard deviations from the group mean.

Probe 1. The area of the pool was divided into four equal quadrants in order to analyze memory retention. As such, the quadrant where the platform was previously located was labelled "Target". To determine whether the rats learned the location of the platform, their total time in the Target was compared to 15 seconds (i.e., the expected chance value when the total 60 seconds is divided by four quadrants) using a one-sample t-test. These tests revealed that all three meal groups spent more time in the target quadrant than would be expected by chance: 1M, $t(8) = 7.593$, $p < 0.001$, Cohen's d = 2.531, MM, $t(10) = 2.253$, $p = 0.024$, Cohen's d = 0.679, AD $t(9) = 6.341$, $p < 0.001$, Cohen's d = 2.005.

A one-way univariate ANOVA was used to determine if there were any differences across meal groups regarding their total time in the target quadrant, Figure 1B. It was found that there was a significant difference across groups, indicating that not all groups spent a similar amount of time in the target quadrant, $F(2,27) = 3.698$, $p = 0.038$, $\eta^2_p = 0.215$. Post hoc comparisons found that the 1M group spent more time in the target than the MM group, $t(27) = 2.375$, $p = 0.025$. Additionally, the AD group spent more time in the target compared to MM group, $t(27) = 2.274$, $p = 0.031$. No differences were found between 1M and AD performance. It must be noted that the same two rats that were excluded from the acquisition analyses were also excluded from any Probe 1 analyses for the same reason. However, they were included in all the subsequent analyses, as their behaviour was not out of the ordinary.

Massed Training. The average latency to reach the hidden platform in a new location was analyzed across training trial and meal group using an ANOVA. A significant effect of trial was found, such that the rats took less time to reach the platform as the trials progressed, $F(9,290) =$

13.340, $p < 0.001$, $\eta^2_p = 0.293$. There was not a significant effect of meal group, indicating that all rats learned the location of the platform similarly, $F(2,290) = 1.113$, $p = 0.330$, Figure 1C.

Probe 2. To determine whether the rats remembered the location of the platform, their total time in the Target was compared to 15 seconds using a one-sample t-test. These tests revealed that all three meal groups spent more time in the target quadrant than would be expected by chance: 1M, $t(10) = 3.268$, $p = 0.004$, Cohen's d = 0.985, MM, $t(10) = 8.947$, $p < 0.001$, Cohen's d = 2.698, AD, $t(9) = 5.417$, $p < 0.001$, Cohen's d = 1.713.

An ANOVA was used to determine if there were any differences across meal groups in their total time in the target quadrant, Figure 1D. There were no significant differences found across meal groups, indicating that all rats retained the second platform location similarly, $F(2,29) = 0.624$, $p = 0.543$, $\eta^2 p = 0.041$.

ClockLab

Food Anticipatory Activity (FAA). Before the meal manipulation was introduced, the rats showed the majority of activity when the lights were off. Once the meal manipulation began, rats in the 1M and MM group demonstrated activity in the light phase while the AD group did not. Examples of this are shown in Figures 2, 3, 4 respectively. Additionally, the majority of the rats in the 1M group and a small number in the MM group displayed FAA such that there was an increase in locomotor activity in the two hours directly preceding the 10:00 feeding. To calculate FAA, the amplitude was taken from the two hours preceding mealtime (i.e., 8:00 to 10:00) and was divided by the total amount of activity in the 24-hour day to create a ratio. A Kruskal-Wallis non-parametric test with pairwise comparisons was conducted to determine any group effects in FAA magnitude. It was shown that there was a significant group effect, $\chi^2(2) = 9.16$, $p = .010$. Further, the 1M group showed a significantly higher magnitude of FAA when compared to the

AD group, $W = 3.565$, $p = .031$, while the MM group showed similar activity when compared to AD, $W = 3.713$, $p = .024$. However, the 1M and MM group did not significantly differ in total magnitude of FAA, $W = 0.891$, $p = 0.804$, Figure 5A.

Nocturnality. Mean differences in nocturnality were observed among groups similar to the FAA patterns above. A Kruskal-Wallis non-parametric test with pairwise comparisons was conducted and found a significant group effect of nocturnal activity, $\chi^2(2) = 6.36$, $p = .041$, such that the MM group showed significantly less than the AD group, $W = 3.416$, $p = .042$. No other significant group differences were found, Figure 5B.

Discussion

The present study examined the effect of the FEO on hippocampal-dependent learning and memory. This was done using a novel food manipulation, such that three different feeding schedules were investigated simultaneously as opposed to previous studies that used two (Lukoyanov et al., 2002; Wall et al., 2019). This included rats that were fed once daily and should have access to the FEO (i.e., 1M), those that were fed multiple and unpredictable times throughout the light cycle and should not have access to the FEO (i.e., MM), and finally those that had constant access to food 24 hours a day as a control group (i.e., AD). After allowing the rats an adequate amount of time to entrain to their feeding schedules (i.e., three weeks), their learning and memory retention were assessed using the MWM task.

Acquisition of the hidden platform version of the WMM was assessed first during the four days of training, and again during a single day of massed training. It was found that all rats learned the task over training days (i.e., during four-day training) and trials (i.e., during massed training), as shown by a significant decrease in latency to the platform. There were no

differences across groups, suggesting that the feeding schedules did not have an impact on learning.

To determine whether the rats retained the location of the hidden platform during acquisition and massed training, a no-platform probe was conducted after both phases of training. Probe 1 was given after the four days of acquisition training, while Probe 2 was given after massed training. For both probes, all rats spent more time in the target quadrant than expected by chance. For Probe 1, the total time spent in the target was compared between the groups and it was found that the 1M and AD group both spent more time in the target compared to the MM group. While the 1M group spent the most time in the target, this number was not significantly different than the AD. Finally, for Probe 2, there were no group differences between the total time spent in the target.

The results for the first probe, were as expected – the 1M rats performed significantly better that the MM rats. Interestingly, there were no differences between the 1M and AD group, suggesting that multiple, unpredictable meals resulted in impaired performance. However, following massed training, this effect was not seen as there were no longer any differences between groups on the probe test.

Locomotor activity was recorded throughout the experiment to determine whether the rats demonstrated FAA, which is an increase in activity directly proceeding mealtime that can indicate the presence of the FEO. As predicted, the 1M group showed FAA (increased activity in the two hours preceding mealtime—i.e., 8:00 to 10:00) indicating that they had access to the FEO. Unexpectedly, a number of MM rats also demonstrated FAA. One reason could be that the MM group was experiencing CRD due to their light-phase feedings, as shown by scattered activity during the light and dark phase. Activity was shown to gradually occur earlier each day

suggesting that their feedings were creating a shift from a nocturnal to diurnal rhythm. As well, the MM group showed significantly lower levels of nocturnality compared to the AD group. Since diurnal animals are awake during the light phase, it would not be unusual for them to show activity during the given hours (i.e., 08:00 to 10:00). Second, social interaction and environmental patterns may have acted as zeitgebers for this group. Just as our body can entrain to lighting and meals, it can also entrain to the interaction with other organisms and the things happening in their surroundings (Aschoff et al., 1971). Although the MM group was not receiving meals at the same time as the 1M, they were still able to see and hear through the cages and perhaps recognized this pattern. This would have allowed for the presence of activity. However, this is not likely considering that the AD group was also in the colony room but did not show FAA.

While this study suggested that multiple, unpredictable meals have a determinantal effect on memory for the location of the platform, it is unknown if this result is due to the effects on the FEO or because of the switch to diurnality in this group. Therefore, it is necessary to replicate this study with all feedings occurring during the rat's active period (i.e., dark phase).

Study 2: Reverse Lighting

To determine if the impaired performance of the MM rats in the first probe was due to negative effects of disrupting the FEO or because of changes in nocturnality, we replicated the previous study but the 1M and MM rats were fed during the dark phase when rats typically eat. To do this, the three groups (1M, MM, and AD) were housed under reverse lighting conditions with lights on at 19:00 and off at 7:00. The feeding times were the same as in Study 1; however, the lights were now off during feeding times. Rats were trained on the MWM and activity was monitored as is Study 1.

If the worsened performance of the MM group in Study 1 was due to the disrupted FEO, then we would again expect the MM group to spend less time in the target quadrant in this study. If, however, the worsened performance of the MM group in Study 1 was due to decreased nocturnality and therefore impaired LEO, then we would expect performance of the MM group to be similar to that of the 1M and AD group in this study.

The 1M group was expected to perform the best due to their consistent mealtimes. However, it was unclear whether the AD group would perform similar to the 1M group, as they also have the ability to activate the FEO depending on their eating habits (Loh et al., 2015). Finally, it was expected that the MM group would exhibit similar nocturnality compared to the 1M and AD group since their feedings were administered during the dark phase.

Method

The methodological details for Study 1 were replicated for Study 2, with a few exceptions. First, there were 16 rats in total—12 in wheels and four in conventional cages with motion sensors. There were six rats in the 1M group, five in the MM group, and five in the AD group. As well, when the food manipulation began, so did the lighting manipulation. The rats were put on a reverse lighting schedule such that the light phase was from 19:00 to 7:00. Finally, all MWM testing was conducted using red light, and rats were transported using a black-out curtain to avoid light confounds.

Results

Morris Water Maze

Acquisition Training. The average latency to reach the hidden platform was analyzed across training day and meal group using an ANOVA, Figure 6A. A significant effect of day was found, such that the rats took less time to reach the platform as the days progressed, $F(3,500) = 58.919$,

$p < 0.001$, $\eta^2 p = 0.259$. There was not a significant effect of meal group, showing that all rats learned the location of the platform similarly, $F(2,500) = 0.333$, $p = 0.717$.

Probe 1. To determine whether the rats remembered the location of the platform, their total time in the Target was compared to 15 seconds using a one-sample t-test. These tests revealed that the 1M, $t(5) = 2.916$, $p = 0.017$, Cohen's d = 1.191, and MM groups, $t(4) = 3.183$, $p = 0.017$, Cohen's d = 1.423, spent more time in the target quadrant than would be expected by chance. The AD group, however, did not spend significantly more time in the target, $t(4) = 0.7914$, $p = 0.237$, Cohen's d = 0.354.

An ANOVA was used to determine if there were any differences across meal groups regarding their total time in the target quadrant. There were no significant differences found across meal groups, indicating that all rats retained the platform location similarly, $F(2,13) = 0.4856$, $p = 0.626$, Figure 6B.

Massed Training. The average latency to reach the hidden platform in a new location was analyzed across training trial and meal group using an ANOVA. A significant effect of trial was found, such that the rats took less time to reach the platform as the trials progressed, $F(9,130) = 7.361$, $p < 0.001$, $\eta^2 p = 0.300$. There was not a significant effect of meal group, indicating that all rats learned the location of the platform similarly, $F(2,130) = 0.856$, $p = 0.427$, Figure 6C.

Probe 2. To determine whether the rats remembered the location of the platform, their total time in the Target was compared to 15 seconds using a one-sample t-test. These tests revealed that all three meal groups spent more time in the target quadrant than would be expected by chance: 1M, $t(5) = 3.809$, $p = 0.006$, Cohen's d = 1.555, MM, $t(4) = 2.762$, $p = 0.025$, Cohen's d = 1.235, AD, $t(4) = 4.314$, $p = 0.006$, Cohen's d = 1.929.

An ANOVA was used to determine if there were any differences across meal groups regarding their total time in the target quadrant, Figure 6D. There were no significant differences found across meal groups, indicating that all rats retained the second platform location similarly, $F(2,13) = 0.3176$, $p = 0.733$, $\eta^2 p = 0.0466$.

ClockLab

Food Anticipatory Activity. A one-way ANOVA was conducted to examine FAA across groups. While there were differences among the food groups, 1M: $M = 29.8$, $SE = 2.94$, MM: $M = 26.6$, $SE = 3.09$, AD: $M = 22.6$, $SE = 4.60$, they were not significant, $F(2,9) = 0.813$, $p = 0.488$. See Figures 7, 8, and 9 for each group's exemplar actigram, respectively, and Figure 10A for FAA values.

Nocturnality. A one-way ANOVA was conducted to determine any group differences among nocturnality. No significant differences were found between groups, $F(2,9) = 0.457$, $p = 0.654$, Figure 10B.

Discussion

The current study investigated the influence of the FEO on learning and memory as a continuation of Study 1. Similar to the previous study, the current study used three different feeding schedules to investigate the effects of manipulation to the FEO and monitored locomotor activity to determine the presence of FAA. In contrast to Study 1, this study implemented a reverse lighting schedule with lights on at 19:00 and off at 7:00. Once rats were given an adequate amount of time to entrain to their lighting and feeding schedules, their learning and memory were tested using the MWM task like Study 1.

Acquisition of the hidden platform version of the MWM was assessed over the four training days and again across the ten massed training trials. There were no differences between

groups in either the initial training or massed training, indicating that all groups learned the location similarly and the feedings did not have an influence on learning.

On the first no-platform probe, both the 1M and MM groups spent more time in the target quadrant than expected by chance. Surprisingly, the AD group did not spend significantly more time in the target quadrant. This was very unexpected given that these rats were identical to the AD group in the first study. The only difference between these groups was that in the second study, the MWM was conducted under red light, whereas in the first study regular lighting was used. However, it is unclear why this would have had an effect – especially since the 1M and MM did do better than chance on the probe trial. Furthermore, there were no group differences in the amount of time in target during the first probe. As in Study 1, all groups spent more time in the correct quadrant in Probe 2 and there were no group differences.

It was expected that the 1M group would perform well on the MWM since their consistent meals should activate the FEO. This hypothesis was supported by the results of both probe trials. However, the MM group also performed above chance during Probe 1 and both the MM and AD groups performed above chance for Probe 2. Although an organism can use several zeitgebers (e.g., food availability, temperature, social influence) to entrain their CR, light has repeatedly been shown to be the most robust zeitgeber. Refinetti (2015) compared the robustness of light and food as entrainment tools among mice by looking at locomotor activity. They found that under a 12:12 LD cycle, one hundred percent of the mice entrained to the lighting schedule with their activity concentrated in the dark phase. When using a single consistent meal as a zeitgeber, it was found that only 44% of mice showed entrainment; however, they did show a high degree of FAA. This shows that although food does act as an entrainment tool, lighting continues to be the most robust tool. Thus, it is likely that the lack of memory deficit found

among the MM group was due to the overwhelming influence of the LEO in comparison to the FEO. Although the MM group had inconsistent meals, this manipulation did not affect memory because the feeding was during the inactive period. To address this, future research should manipulate the FEO in the absence of the LEO, such as in constant dark or light. This is explored in Study 3.

Additionally, there were similar ceiling effects in training demonstrated during this study compared to the previous study. The most apparent reason for the results is that the rats were likely over-trained in the MWM thus creating a ceiling effect. When looking at Figure 1A, 1C, 6A, and 6C, it is evident that most of the learning occurred between Day 1 and Day 2 during the four-day training and between trials one to six during massed training. By overtraining, it is possible that long-lasting changes occurred in the brain that allowed for greater memory retention, as evidenced by Gomez-Padilla et al., (2020). Specifically, they found that over-trained animals on the MWM produced an increase in the density of thorny excrescences found in the CA3 basal dendrite. The authors suggested that the increase in density would allow for more connections among these clusters and the surrounding neurons, therefore increasing activity in this region of the brain and in turn, aiding in long-term memory retention. This ceiling effect could be relieved in one of two ways. First, the amount of training for the MWM task could be reduced. As mentioned, most of the learning occurred after approximately eight trials, indicating that further training is not necessary. Additionally, a different cognitive task could be used instead of the MWM. There are many tasks in the literature that target spatial (i.e., hippocampal-dependent) memory, and more that are emerging. Tasks such as the radial arm maze (RAM), Barnes maze task (BM), and spatial versions of the novel object recognition test are examples of alternates that could be used to determine if the ceiling effects were created due

to the task itself (i.e., MWM) or simply the length of training (Gawel et al., 2019; Shukitt-Hale et al., 2004).

Locomotor activity was recorded throughout the study to examine FAA, if present, as a way of determining the presence of the FEO. While it was predicted that only the 1M group would display FAA, it was found that all groups demonstrated an increase of activity from 8:00 to 10:00. The 1M group had a consistent and high amount of activity during those hours, although this was not significantly higher than the other groups. As well, there were no differences in nocturnality among the groups indicating that all the rats were primarily active during the dark phase of the LD cycle. These results are likely due to social or environmental pattern entrainment like those found in Study 1. The presence of a researcher at 10:00 may not have been a salient cue for the AD group in the previous study because they were entering the room during the light cycle, which is when the rats would normally be inactive. However, for Study 2, the researcher was entering the room during the dark phase. Perhaps this was more salient considering the rats were active and awake, thus noticing the environmental cues more. As mentioned in the discussion of Study 1, future studies should keep the meal groups separated to prevent the entrainment of environmental cues.

Studies such as this are crucial for understanding the role of external stimuli on the circadian rhythm and in turn, an organism's behavior. Although the current study did not provide conclusive evidence surrounding the effect of mealtime on learning and memory, it did give insight into lighting effects. Specifically, this study showed that lighting remains the most robust zeitgeber, often overshadowing the influence of other zeitgebers such as meal timing. Thus, Study 3 implemented a manipulation that ensured the effects of the FEO can be investigated without influence from the LEO.

Study 3: Constant Darkness

The combined results of Studies 1 and 2 suggest that in the presence of a functional LEO, the effects of the FEO are minimal. The purpose of the present study was twofold. First, we determined the effect of the FEO when the LEO input is absent (i.e., constant darkness). Second, we made the task more difficult to determine if the FEO is having an effect but is masked by the ceiling effects shown in the first two studies.

In Study 2, the MM group remembered the location of the platform in both probes as well as the 1M group. This suggests that the impaired performance in the first study was likely due to the disrupted LEO induced by increased diurnality. Therefore, it would appear that as long as the LEO is functional, the FEO does not have an effect. By housing the rats in constant darkness, we will be able to determine the effects of the FEO.

Based on the results of Study 1 and 2, it was expected that all the standard lighting (i.e., LD) rats would learn the task since they have access to the LEO. For the rats in the constant darkness (i.e., DD) group, it was expected that the DD-1M rats would learn the task, the DD-MM rats would not, and the DD-AD most likely would due to free running rhythms (but this will be interesting to confirm). Without overtraining, it would be easier to see these effects. Because the tests are more sensitive, we hypothesize that if there is an additive effect of having both the LEO and FEO (i.e., the LD-1M group), that we will be able to see this. Additionally, it was expected that in the absence of lighting cues, the AD group would display the highest amount of nocturnal activity. This is because the 1M and MM groups will not be fed throughout the entire 24 hours (i.e., they are fed during the daytime), thus their activity may cluster during this half of the day. Finally, it was expected that the 1M group would exhibit robust FAA due to their consistent mealtime, while the MM and AD groups would not. Overall, it was expected that the

LD-1M group would show the best performance due to the activation of the FEO and the LEO, while the DD-MM group was expected to show the worst performance due to disruption of the FEO and LEO.

Method

The methodological details for Study 1 were replicated for Study 3, with a few exceptions. First, there were 48 rats in total, all of which were housed in conventional cages with motion sensors. There were eight rats in each of the six groups. Next, the MWM acquisition and retention testing followed a new schedule. The rats had a single day of training with a total of 10 trials each. Twenty-four hours later they completed a 60-second probe. After another five days, they completed a second probe. Additionally, when the food manipulation began, so did the lighting manipulation. Half of the rats were in a standard lighting group with the light phase from 7:00 to 19:00. The other half of the rats were put into constant darkness. The rats were fed during the light phase as in in Study 1. Finally, all MWM testing was conducted using red light for the rats in the constant darkness condition and they were transported using a black-out curtain to avoid light confounds.

Results

Morris Water Maze

Massed Training. The average latency to reach the platform was analyzed across trials as well as meal and lighting group using an ANOVA. A significant effect of trial was found, such that the rats took less time to reach the platform as the trials progressed, $F(9,470) = 9.62$, $p < 0.001$, $\eta^2p = 0.156$. There was not a significant effect of meal group, $F(2,474) = 1.343$, $p = 0.262$, $\eta^2p = 0.005$, indicating that the meal groups learned the location of the platform similarly. However, a significant effect of lighting group was observed, $F(1, 474) = 11.653$, $p < 0.001$, $\eta^2p = 0.024$.

Post hoc tests revealed differences between DD-MM and LD-1M, $t(474) = -2.930$, $p = 0.041$, as well as DD-MM and LD-AD, $t(474) = -3.263$, $p = 0.015$, such that the DD-MM performed significantly worse in both comparisons, Figure 11A. No significant interaction was found, $F(2,474) = 0.294$, $p = 0.746$, $\eta^2 p = 0.001$. No other differences were found.

Probe 1. To determine whether the rats remembered the location of the platform after a delay of 24 hours, their total time in the Target was compared to 15 seconds using a one-sample t-test. All groups spent significantly more time in the target quadrant compared to chance: DD-1M, $t(7) = 3.91$, $p = 0.003$, Cohen's d = 1.38, DD-MM, $t(7) = 3.64$, $p = 0.004$, Cohen's d = 1.29, DD-AD, $t(7) = 2.54$, $p = 0.019$, Cohen's d = 0.0899, LD-1M, $t(7) = 4.91$, $p < 0.001$, Cohen's d = 1.74, LD-MM, $t(7) = 3.59$, $p = 0.004$, Cohen's d = 1.27, and LD-AD, $t(7) = 3.99$, $p = 0.003$, Cohen's d = 1.41.

An ANOVA was used to determine if there were any differences across lighting or meal group in their total time in the target quadrant, Figure 11B. There were no significant differences found across meal groups, $F(2,42) = 1.886$, $p = 0.164$, $\eta^2 p = 0.080$, or lighting group, $F(1,42) = 0.913$, $p = 0.345$, $\eta^2 p = 0.019$, indicating that all rats retained the platform location similarly. As well, the interaction was not significant, $F(2,42) = 0.378$, $p = 0.688$, $\eta^2 p = 0.016$.

Probe 2. To determine whether the rats remembered the location of the platform after a delay period of five days, their total time in the Target was compared to 15 seconds using a one sample t-test. All groups, except one, spent significantly more time in the target quadrant compared to chance: DD-1M, $t(7) = 2.532$, $p = 0.020$, Cohen's d = 0.8953, DD-AD, $t(7) = 2.170$, $p = 0.033$, Cohen's d = 0.7674, LD-1M, $t(7) = 8.176$, $p < 0.001$, Cohen's d = 2.891, LD-MM, $t(7) = 2.459$, $p = 0.022$, Cohen's d = 0.8694, LD-AD, $t(7) = 3.348$, $p = 0.006$, Cohen's d = 1.184. The DD-

MM group did not spend more time in the target quadrant compared to chance, $t(7) = 1.696$, p 0.067, Cohen's d = 0.5997.

An ANOVA was used to determine if there were any differences across lighting or meal group in their total time in the target quadrant, Figure 11C. There were no significant differences found across meal groups, $F(2,42) = 1.007$, $p = 0.374$, $\eta^2p = 0.0421$, or lighting group, $F(1,42) = 3.805$, $p = 0.058$, $\eta^2p = 0.0795$. As well, the interaction was not significant, $F(2,42) = 0.0311$, $p = 0.969$, $\eta^2p = 0.0013$.

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Food Anticipatory Activity. An ANOVA determined that there was not a significant difference in FAA across lighting groups, $F(1,15) = 0.1112$, $p = 0.743$, $\eta^2p = 0.0013$. However, there was a difference across meal groups, $F(2,15) = 35.8051$, $p < 0.001$, $\eta^2p = 0.8202$. Post hoc analyses revealed that the MM group had a higher magnitude of FAA compared to the AD group, $t(15) = 4.749$, $p < 0.001$, Cohen's d = 2.565, the 1M group had a higher magnitude compared to the AD group, $t(15) = 8.462$, $p < 0.001$, Cohen's d = 4.730, and the 1M group had a higher magnitude compared to the MM group, $t(15) = 4.161$, $p = 0.002$, Cohen's d = 2.165. The lighting and meal group interaction was not significant, $F(2,15) = 0.2924$, $p = 0.751$, $\eta^2p = 0.0067$. Refer to Figures 12 through 17 for exemplar actigrams for each group, and Figure 18A for FAA values.

Nocturnality. An ANOVA was conducted to determine any differences in nocturnality across groups, Figure 18B. It was found that a significant difference occurred across meal groups, $F(2,15) = 15.247$, $p < 0.001$, $\eta^2p = 0.6613$, but not lighting groups, $F(1,15) = 0.6006$, $p = 0.450$, $\eta^2p = 0.0130$. As well, the lighting and meal group interaction was not significant, $F(2,15) = 0.01029$, $p = 0.990$, $\eta^2p = 0.0004$. Post hoc analyses for the meal group effect found that the AD group showed significantly more nocturnal activity compared to the MM group, $t(15) = 5.1406$,

$p < 0.001$, Cohen's d = 2.7762, and the 1M group, $t(15) = 4.5198$, $p = 0.001$, Cohen's d = 2.5266. No differences were shown between the 1M and MM group.

Discussion

The current study was the final experiment of this investigation on the role of FEO on learning and memory. Specifically, this study utilized constant darkness to eliminate the influence of the LEO so that we could investigate the FEO independently.

The results of the MWM agree with previous findings that hippocampal dependent learning is impaired among rats with disrupted oscillators, such as in the DD-MM group. For example, Wall et al. (2019) investigated the influence of FEO on learning and found that the MM group performed worse on a TPL task when compared to the 1M group. As well, Craig and McDonald (2008) found that rats with chronic LEO disruption took almost twice as long to find the hidden platform and had much longer path lengths in a MWM acquisition task. In Study 1 and Study 2 of the current research, there were no differences in acquisition among groups. However, they were subjected to a much longer training period than the rats in this study, indicating that LEO and FEO disruption may impair massed learning but not distributed learning. Alternatively, it could be that an additive effect of LEO and FEO, as seen in the LD-1M group (although not significant), may improve learning. To determine whether these results are the product of an impairment of one group, or the improvement of the other, future studies should subject the rats to a MWM task before manipulations are introduced to view the baseline performance.

Although it was expected that the DD-MM would perform the worst on both probes, it was found that the memory deficit was only present during the second probe. However, this is the first study in the current collection that observed both short-term and long-term memory

retention. It is not surprising that the DD-MM group displayed a memory deficit on the second probe, as having a delay period between learning and memory retention testing has been shown to produce similar findings (Chang et al., 2009; Ruby et al., 2008). For example, Chang et al. (2009) disrupted the LEO in rats by subjecting them to a sleep deprivation paradigm. They tested these rats on the MWM immediately following sleep deprivation and again the day after, finding that the rats' memory was worse between the first and second retention testing. Similar results were found by Ruby et al. (2008) such that light-disrupted hamsters performed best on a novel object recognition test directly after their learning phase, but performance deteriorated with each additional retention test. Thus, the current results support previous findings stating that rodents with disrupted circadian systems are likely to experience memory deficits as the time since acquisition has increased. It could be expected that the DD-MM group would show an even stronger deficit if the delay period was longer. Future research should continue to administer probe trials long term to determine if other groups would follow the same trend. As well, it would help determine how an additive effect of LEO and FEO (i.e., the LD-1M group) would maintain memory retention over time.

FAA was measured using motion sensors to determine whether the FEO was active. It was expected that the 1M group would show the highest amount of FAA due to their consistent mealtimes while the AD group would show little FAA. Both predictions were supported by the current results. As well, it was found that the MM group did have activity during the two-hour window preceding the 1M's mealtime although it was significantly less activity than shown by the 1M group. This finding was somewhat expected due to experimenter and environmental influence as mentioned in Study 1 and 2. As previously stated, a way to control for this would be to house each group separately so that their activity is not affected by outside influence. Finally,

as expected, the AD group showed more nocturnal activity compared to the other two meal groups. This was predicted as the 1M and MM groups were only being fed during a 12-hour section of the day, while the AD group could eat throughout the entire 24-hours. The design of this study was created prior to the results of Study 2. Thus, it is now apparent that a reverse light cycle versus constant darkness would have been more appropriate for this study to address the nocturnality issue. Future research should replicate the current study but use a reverse light paradigm as opposed to standard lighting.

In the first two studies, acquisition of the hidden platform was assessed over four training days with eight trials each, and then again on a single massed training day with 10 trials. It appeared that this amount of training created a ceiling effect such that all groups learned the location of the platform and plateaued at a short latency. Thus, this study had a single day of massed training with 10 trials to eliminate over-training. The results from the massed training showed that all groups learned the platform location. As well, the DD-MM group showed higher latencies than both the LD-1M and LD-AD groups. However, the total time spent in the target quadrants during both probes was similar to Study 1 and 2, indicating that this task is still too simple. Thus, alternate versions of the MWM task or a different task altogether should be implemented in future studies.

This study investigated the influence of the FEO on learning and memory, independently and together with the LEO. As in previous studies, it was found that consistent mealtimes (i.e., the 1M group) provided a cognitive advantage over inconsistent mealtimes by recruiting the FEO. These results were amplified when the consistent meals were administered under a 12:12 LD cycle and therefore had a functional LEO. Thus, this study provided evidence that these

oscillators are important for optimal learning and memory retention, and that they work together to create an additive effect with an even more robust influence.

General Discussion

The current collection of studies examined whether an active FEO would benefit learning and memory and whether a disrupted FEO impairs these cognitive functions. This was done by comparing three meal groups (1M, MM, and AD) on a hippocampal-dependent task (i.e., MWM) under different lighting conditions.

In Study 1, we were able to show that consistent mealtimes (i.e., the 1M group) provided an advantage for learning and memory while inconsistent, random meals (i.e., the MM group) impaired memory. However, the MM group demonstrated FAA and less nocturnality than expected. Thus, it was not possible to determine what the results of a disrupted FEO would be since the MM group also experienced a disrupted LEO. Thus, Study 2 utilized a reverse light cycle to eliminate the nocturnality issue from Study 1. While this manipulation produced the expected results and no differences in nighttime activity was found among groups, the MM group was still able to retain the location of the platform similarly to the other groups. This provides evidence that when the LEO is functional it is more influential than other oscillators. Since the SCN entrains to the environment via light information, the current findings support that the SCN is the "master clock" of the body. While this is important to confirm, the goal of these studies was to determine the influence that the FEO has on learning and memory. Thus, the next study used constant darkness to eliminate the effects of the LEO on learning and memory so the FEO could be viewed independently. In Study 3, there was an acquisition and retention impairment for rats housed in constant darkness with multiple, inconsistent meals (i.e., DD-MM), indicating that there is an additive effect from the LEO and FEO. Taken together, it was

shown that light remains the most prominent zeitgeber such that the LEO overshadows other oscillators when it is in use. When these oscillators are disrupted, detrimental effects on hippocampal-dependent learning and memory are likely to occur.

Studies such as the current are important for understanding how entrainment tools such as lighting and mealtime have an influence on CRs and memory. Circadian rhythm disruptions are often found among individuals who have mood disorders, cancer, and severe memory conditions such as Alzheimer's disease (Zelinski et al., 2014; Zelinski et al., 2013). Additionally, these disruptions are found among shift workers and frequent travellers, as their lighting schedule is disturbed (Czeisler & Klerman, 1998; Karatsoreos et al., 2011)**.** Thus, it is crucial to understand how these CRDs can influence cognition, as many shift workers are involved in critical work such as healthcare. As well, if it is determined through future research that consistent meals throughout the day provide relief for the detrimental cognitive effects found with a CRD, this FEO-induced regimen can be implemented to improve their day-to-day lives.

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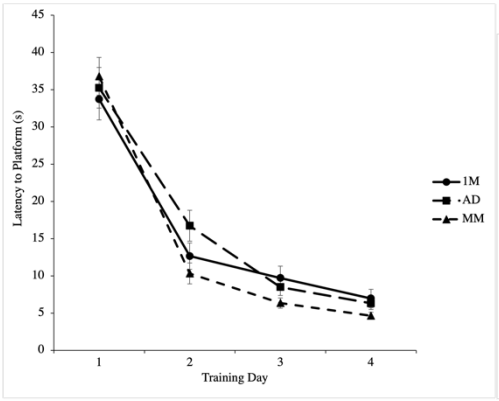
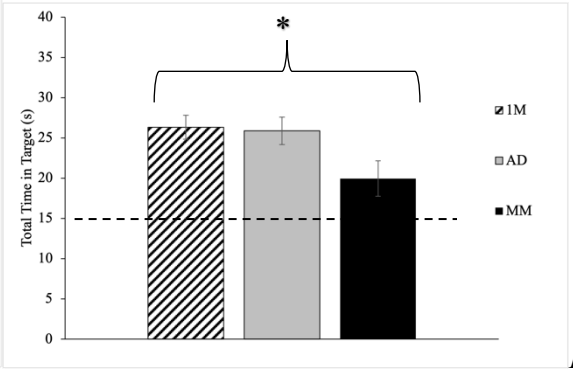
Zelinski, E, L., Tyndall, A, V., Hong, N, S., & McDonald, R, J. (2013). Persistent impairments in hippocampal, dorsal striatal, and prefrontal cortical function following repeated

photoperiod shifts in rats. *Experimental Brain Research, 224*, 125-139. https://doi.org/10.1007/s00221-012-3293

Figure 1: Study 1



A.



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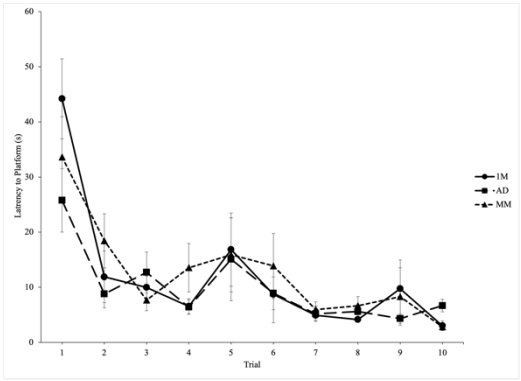

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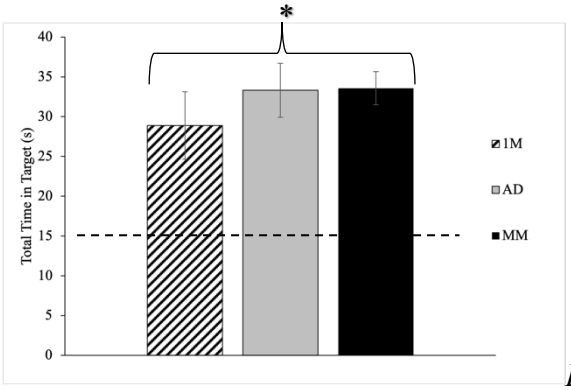
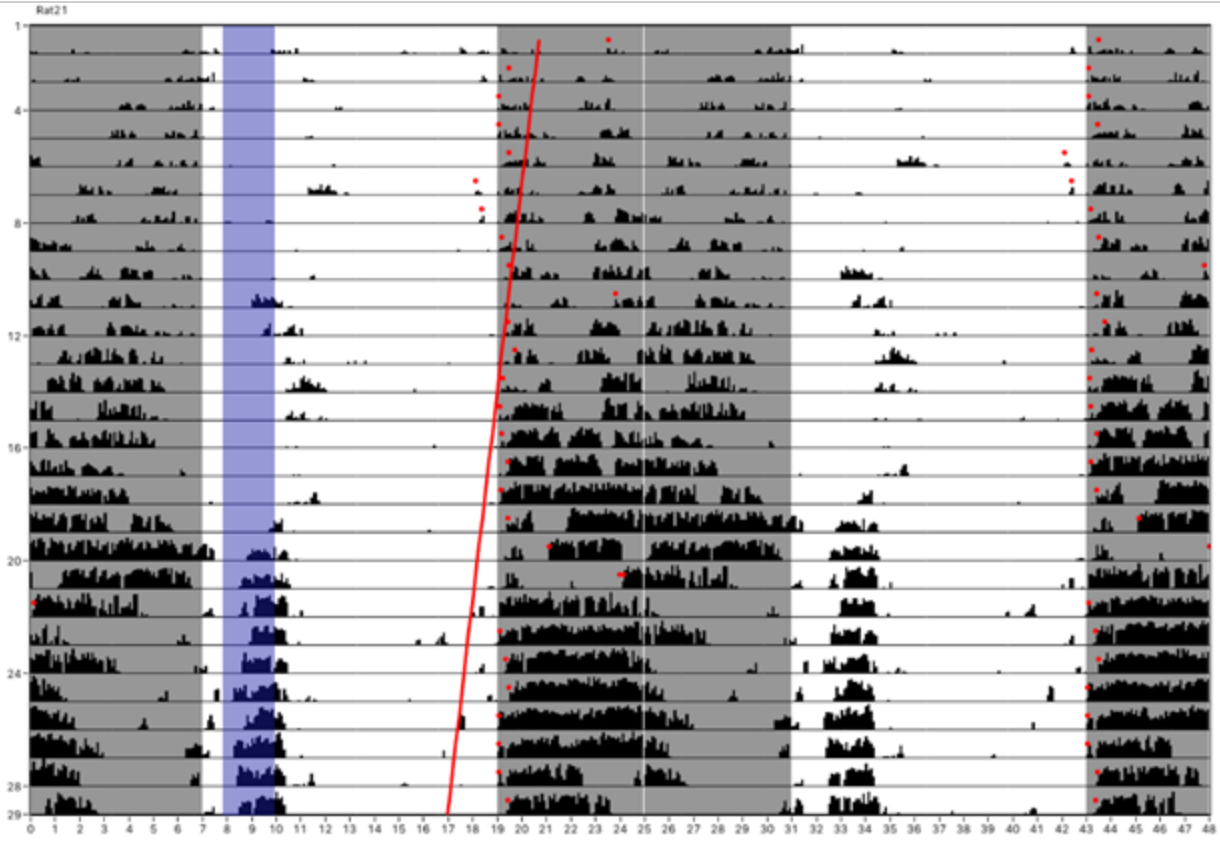


D.

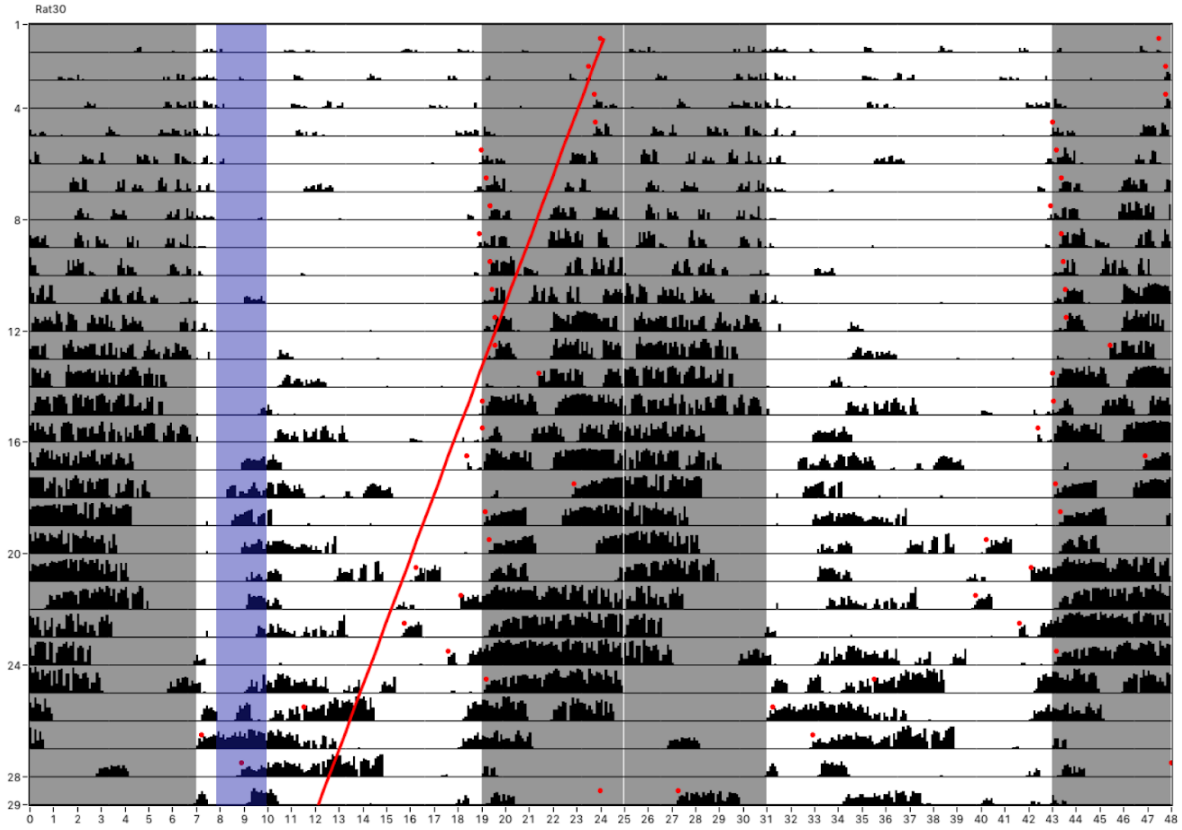
A. *Meal Groups' Mean Latency to Platform (s) During the Four-Day Acquisition Period of the Morris Water Maze Task. Error bars represent SEM. Eight trials were conducted each training day.*
B. *Meal Groups' Total Time (s) Spent in the Target Quadrant of the Morris Water Maze Task for Probe 1. Error bars represent SEM. Rats spent a total of 60 seconds in the maze. Dotted line represents chance level. All groups spent significantly more time in the target than expected by chance. 1M and AD groups spent significantly more time in target than the MM group. Asterisk indicates values significantly different from chance.*
C. *Meal Groups' Mean Latency to Platform (s) During the 10-Trial Massed Training of the Morris Water Maze Task. Error bars represent SEM. All 10 trials were completed in a single day.*
D. *Meal Groups' Total Time (s) Spent in the Target Quadrant of the Morris Water Maze Task for Probe 2. Error bars represent SEM. Rats spent a total of 60 seconds in the maze. Dotted line represents chance level. All groups spent significantly more time in the target than expected by chance. There were no group differences in the total time spent in the target. Asterisk indicates values significantly different from chance.*

Figure 2. Actigram of Locomotor Activity in a 1M Rat Demonstrating FAA.



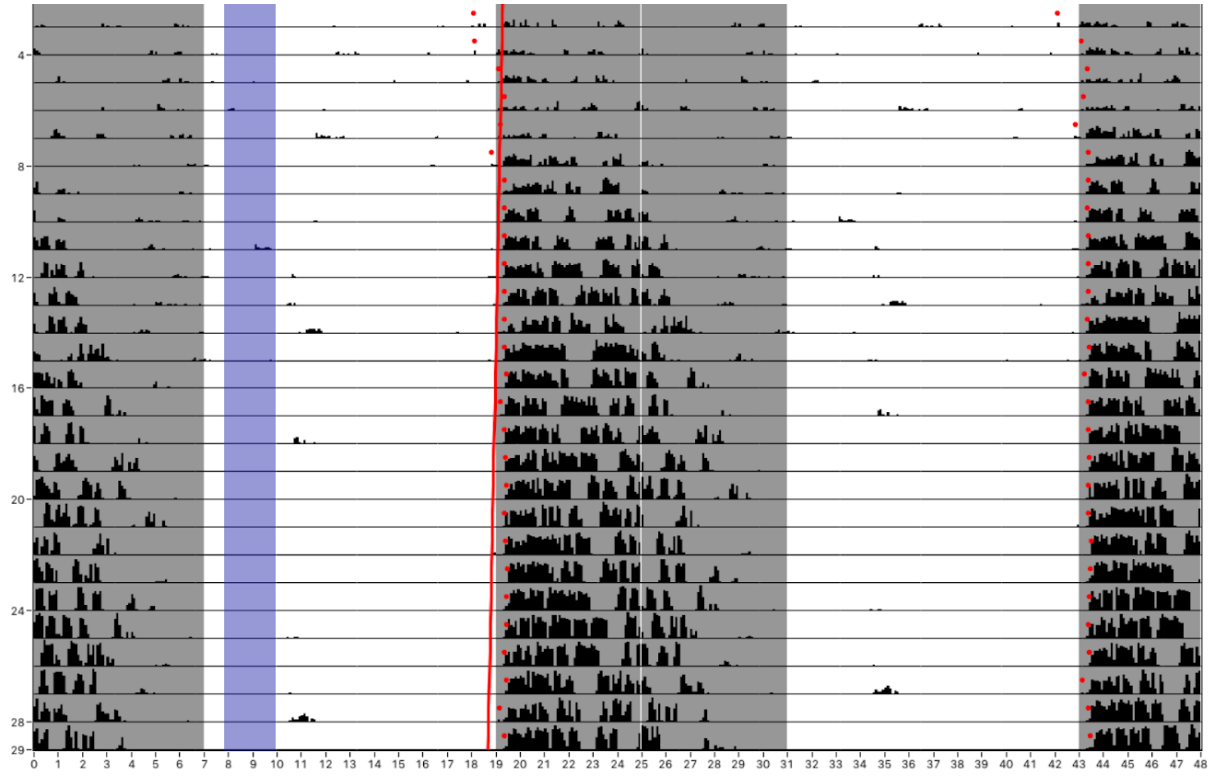
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The blue section highlights 08:00 to 10:00 (i.e., the area where FAA would appear). The red line shows mean time onset of activity.

Figure 3. Actigram of Locomotor Activity in a MM Rat Demonstrating FAA.



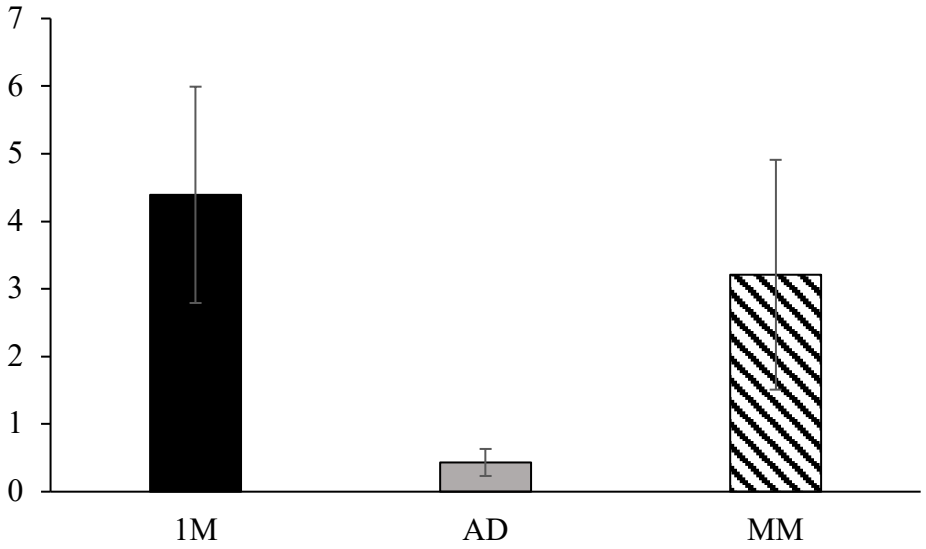
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The blue section highlights 08:00 to 10:00 (i.e., the area where FAA would appear). The red line shows mean time onset of activity.

Figure 4. Actigram of Locomotor Activity in an AD Rat not Demonstrating FAA.

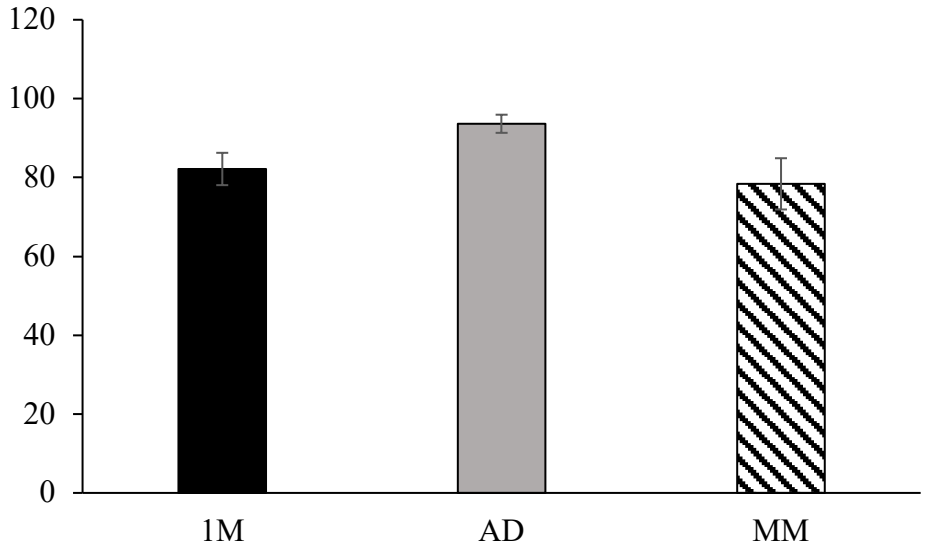


Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The blue section highlights 08:00 to 10:00 (i.e., the area where FAA would appear). The red line shows mean time onset of activity.

Figure 5. Clocklab Analyses: Study 1

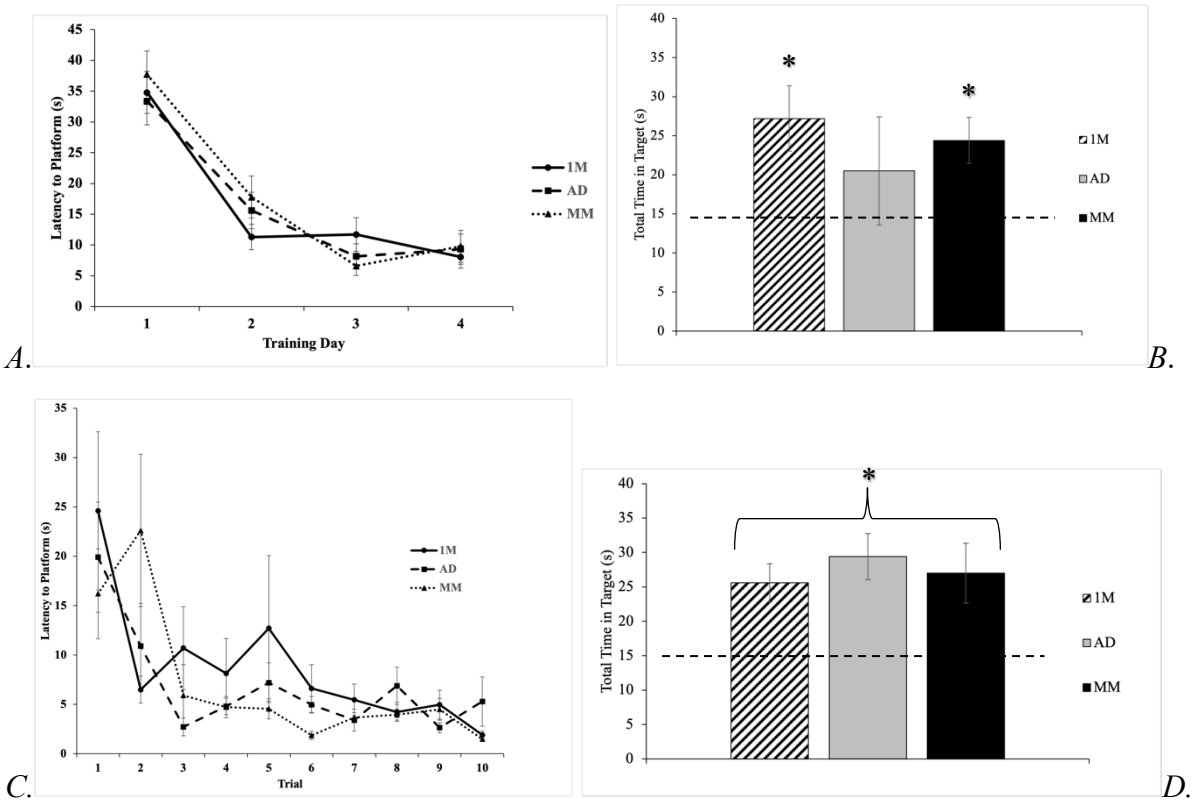


A. Mean FAA Absolute Values with SEM. The 1M group demonstrated significantly higher magnitudes of FAA compared to the AD group.



B. Mean Nocturnality Absolute Values with SEM. The AD group showed significantly more nighttime activity compared to the MM group.

Figure 6: Study 2

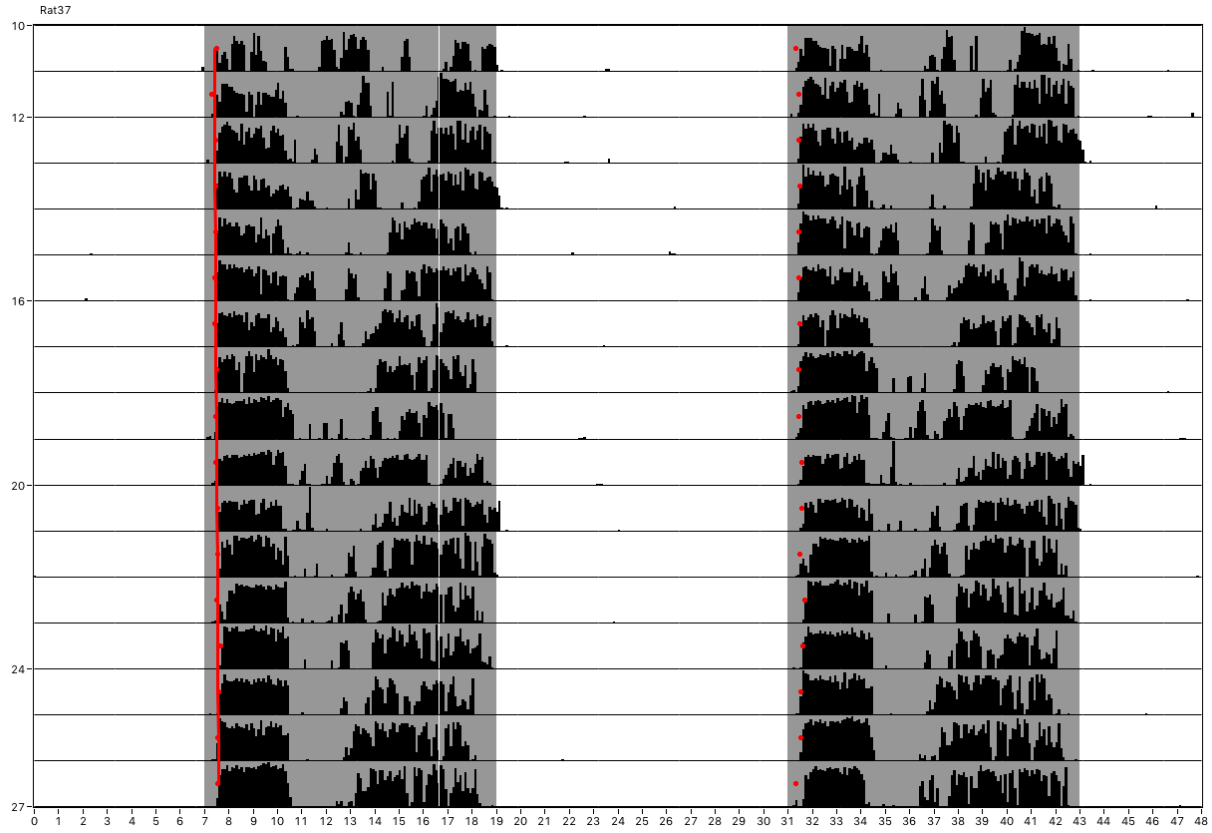


A. *B.*

C. *D.*

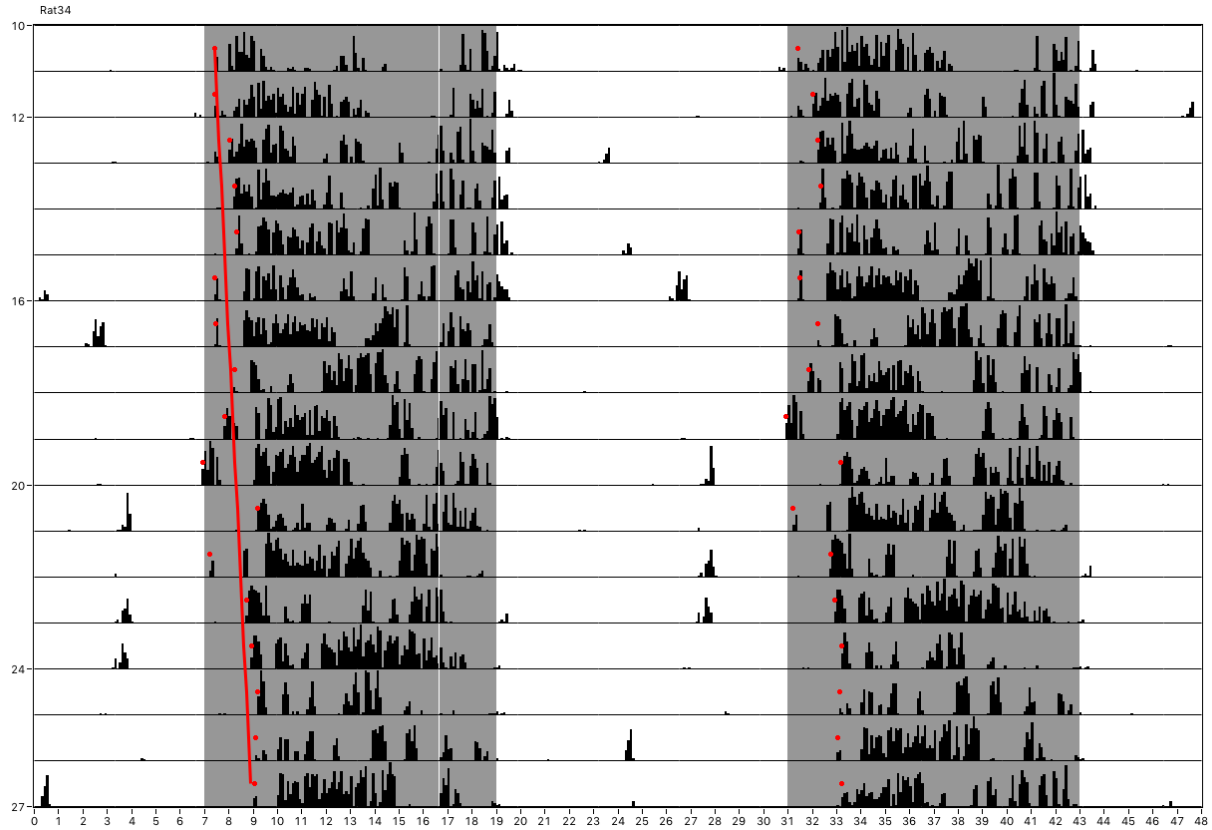
A. *Meal Groups' Mean Latency to Platform (s) During the Four-Day Acquisition Period of the Morris Water Maze Task. Error bars represent SEM. Eight trials were conducted each training day.*
B. *Meal Groups' Total Time (s) Spent in the Target Quadrant of the Morris Water Maze Task for Probe 1. Error bars represent SEM. Rats spent a total of 60 seconds in the maze. Dotted line represents chance level. 1M and MM group spent significantly more time in the target than expected by chance. There were no group differences in the total time spent in the Target. Asterisk indicates values significantly different from chance.*
C. *Meal Groups' Mean Latency to Platform (s) During the 10-Trial Massed Training of the Morris Water Maze Task. Error bars represent SEM. All 10 trials were completed in a single day.*
D. *Meal Groups' Total Time (s) Spent in the Target Quadrant of the Morris Water Maze Task for Probe 2. Error bars represent SEM. Rats spent a total of 60 seconds in the maze. Dotted line represents chance level. All groups spent significantly more time in the target than expected by chance. There were no group differences in the total time spent in the target. Asterisk indicates values significantly different from chance.*

Figure 7. Actigram of Locomotor Activity in a 1M Rat Demonstrating FAA.



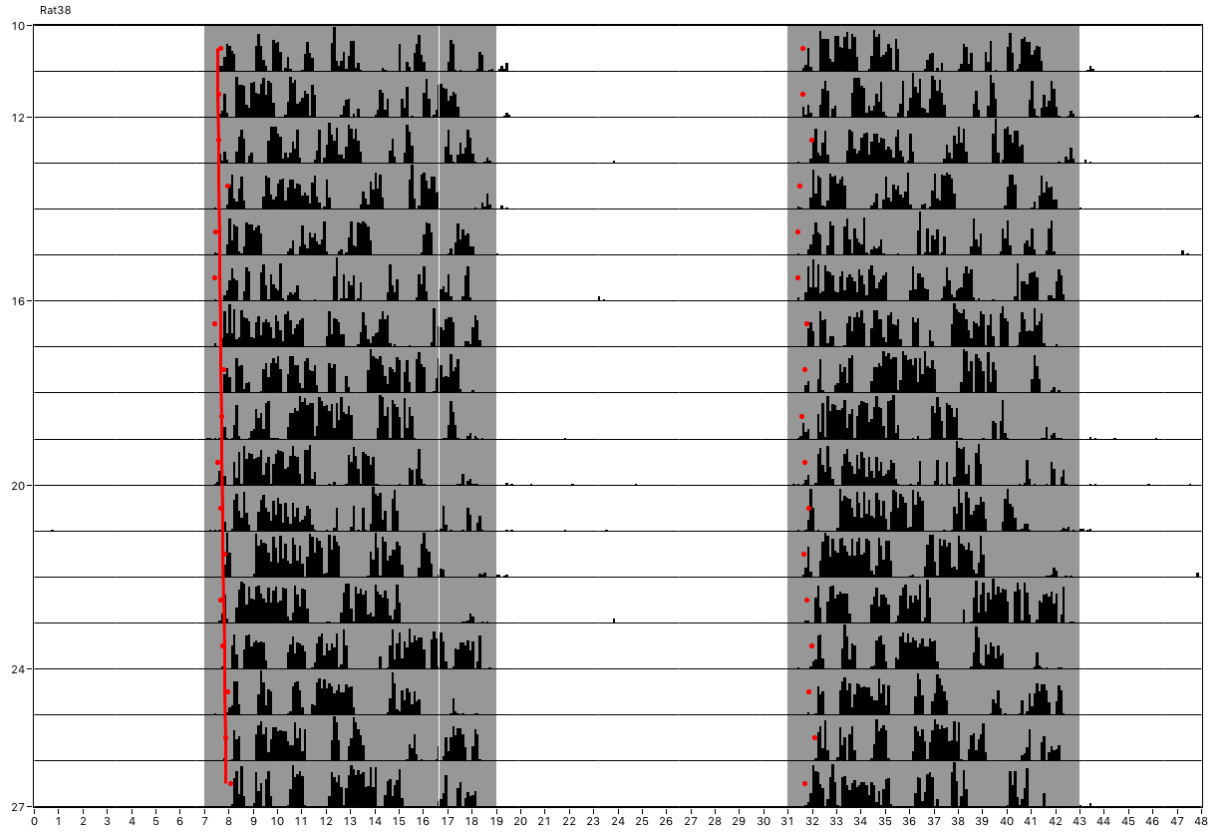
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The red line shows mean time onset of activity.

Figure 8. Actigram of Locomotor Activity in a MM Rat Demonstrating FAA.



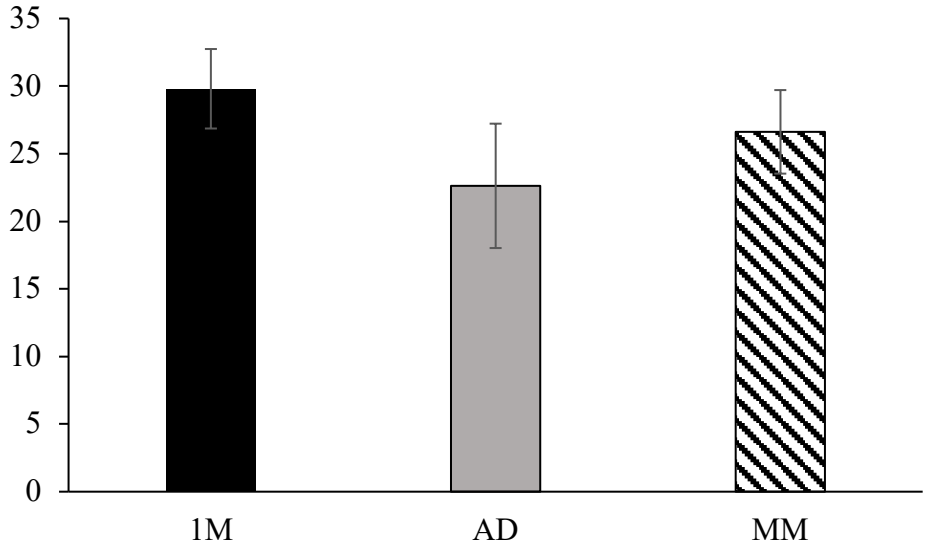
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The red line shows mean time onset of activity.

Figure 9. Actigram of Locomotor Activity in an AD Rat Demonstrating FAA.

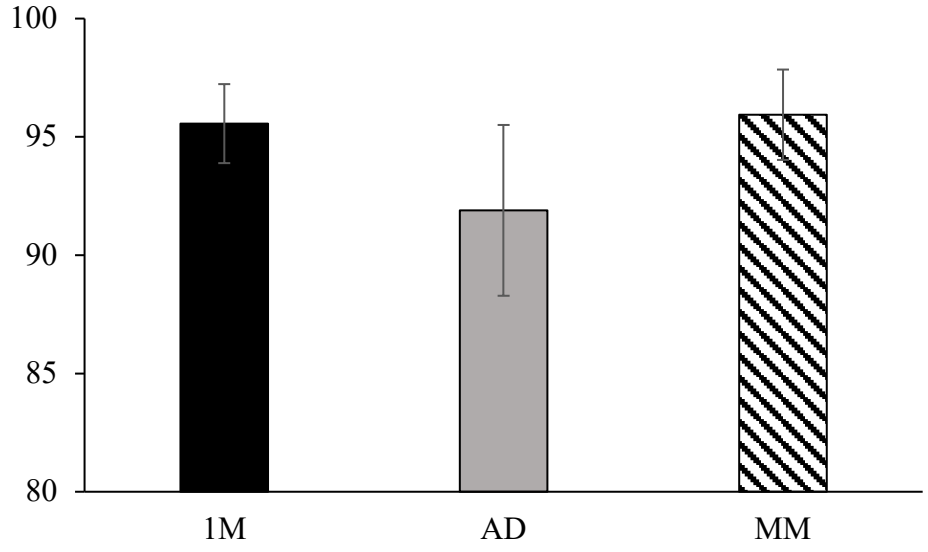


Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The red line shows mean time onset of activity.

Figure 10. Clocklab Analyses: Study 2.

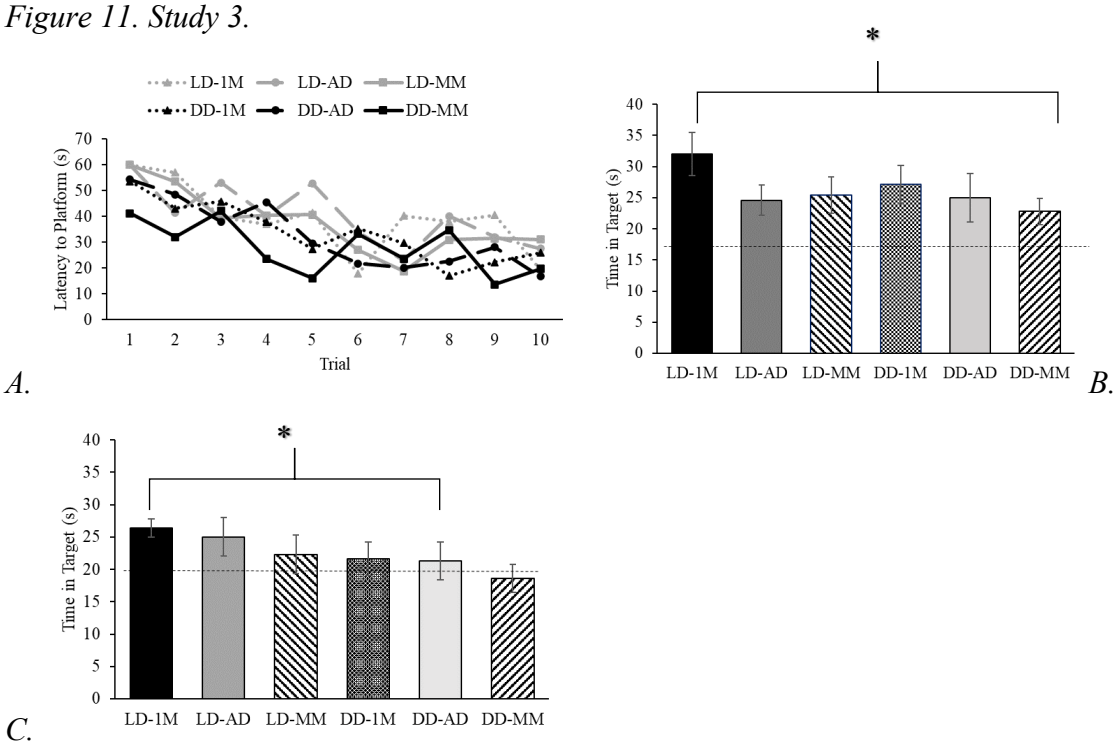


A. Mean FAA Absolute Values with SEM. No differences between groups.



B. Mean Nocturnality Absolute Values with SEM. No differences between groups.

Figure 11. Study 3.



A. *B.*

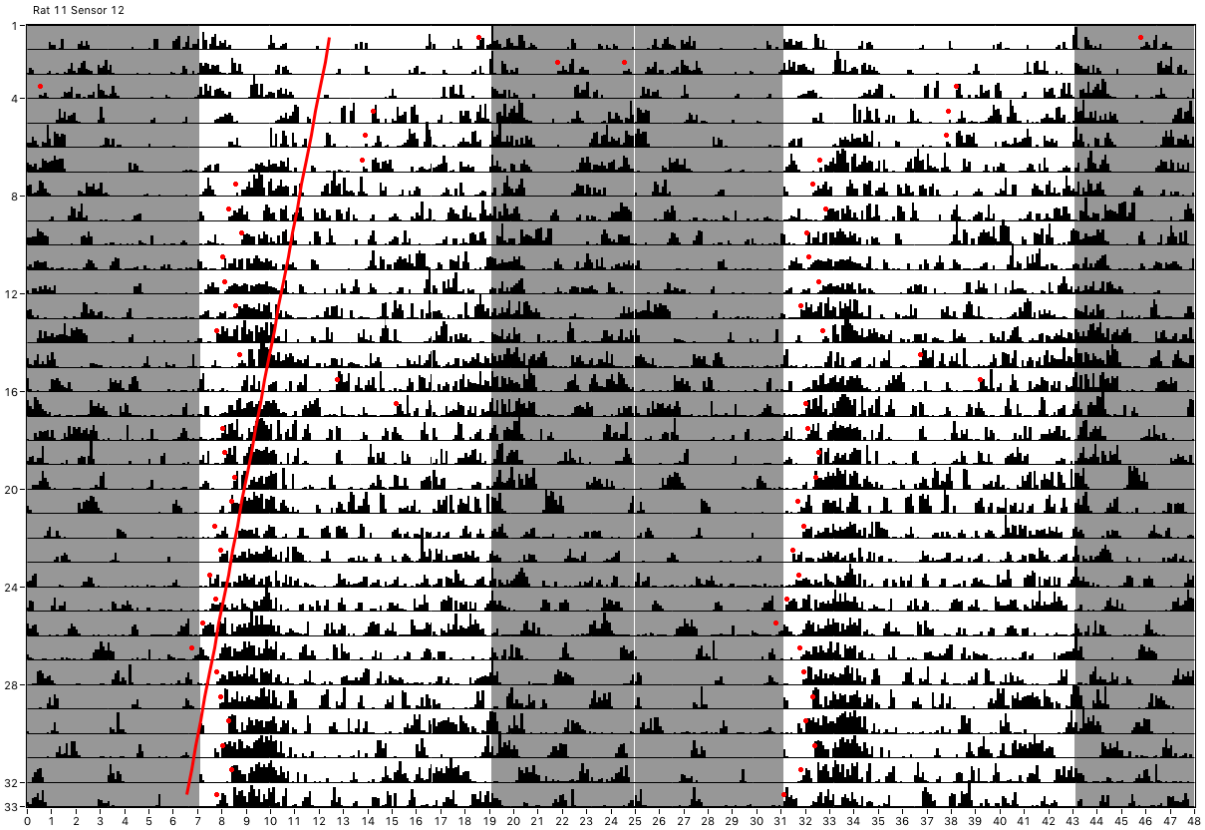
C.

A. Groups' Mean Latency to Platform (s) During the 10-Trial Massed Training of the Morris Water Maze Task. Error bars represent SEM. All 10 trials were completed in a single day.

B. Meal and Lighting Groups' Total Time (s) Spent in the Target Quadrant of the Morris Water Maze Task for Probe 1. Error bars represent SEM. Rats spent a total of 60 seconds in the maze. Dotted line represents chance level. All groups spent significantly more time in the target than expected by chance. There were no group differences in the total time spent in the Target. Asterisk indicates values significantly different from chance.

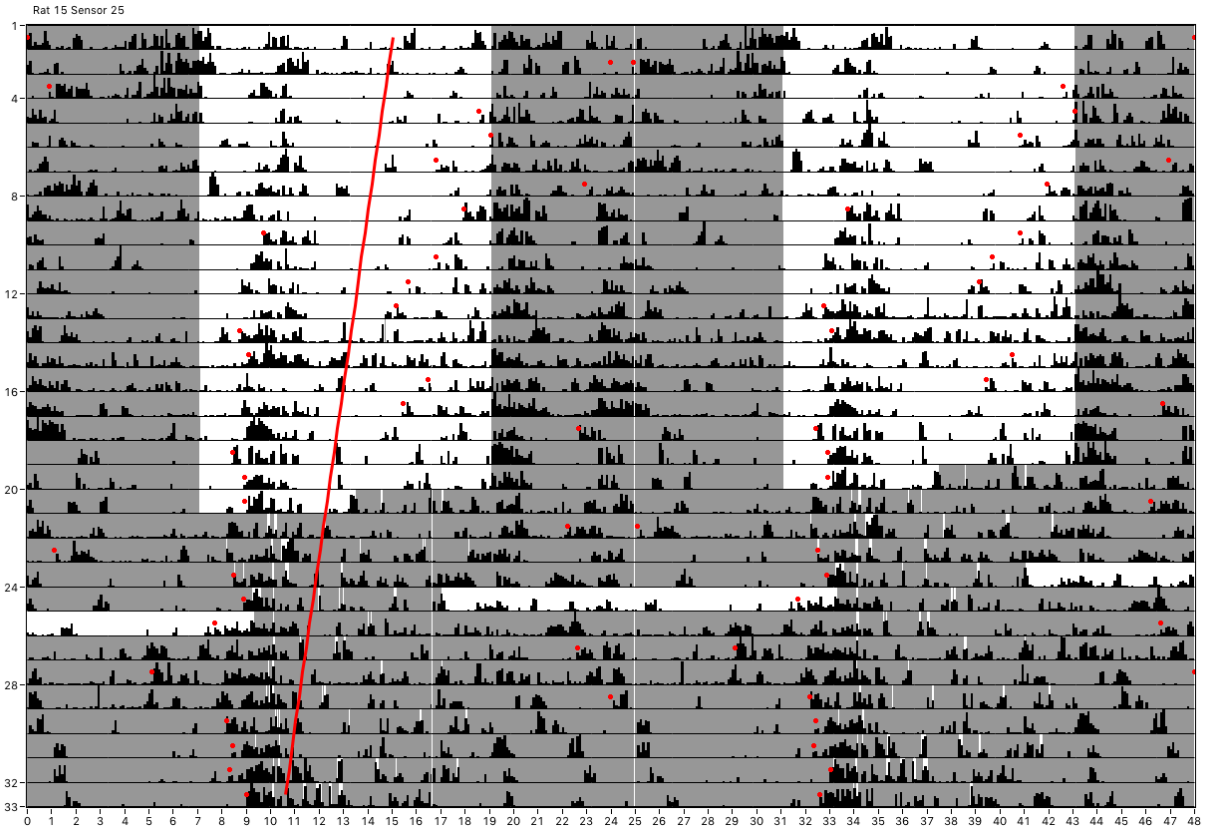
C. Meal and Lighting Groups' Total Time (s) Spent in the Target Quadrant of the Morris Water Maze Task for Probe 2. Error bars represent SEM. Rats spent a total of 60 seconds in the maze. Dotted line represents chance level. All groups spent significantly more time in the target than expected by chance except for the DD-MM group. There were no group differences in the total time spent in the Target. Asterisk indicates values significantly different from chance.

Figure 12. Actigram of Locomotor Activity in a LD-1M Rat Demonstrating FAA.



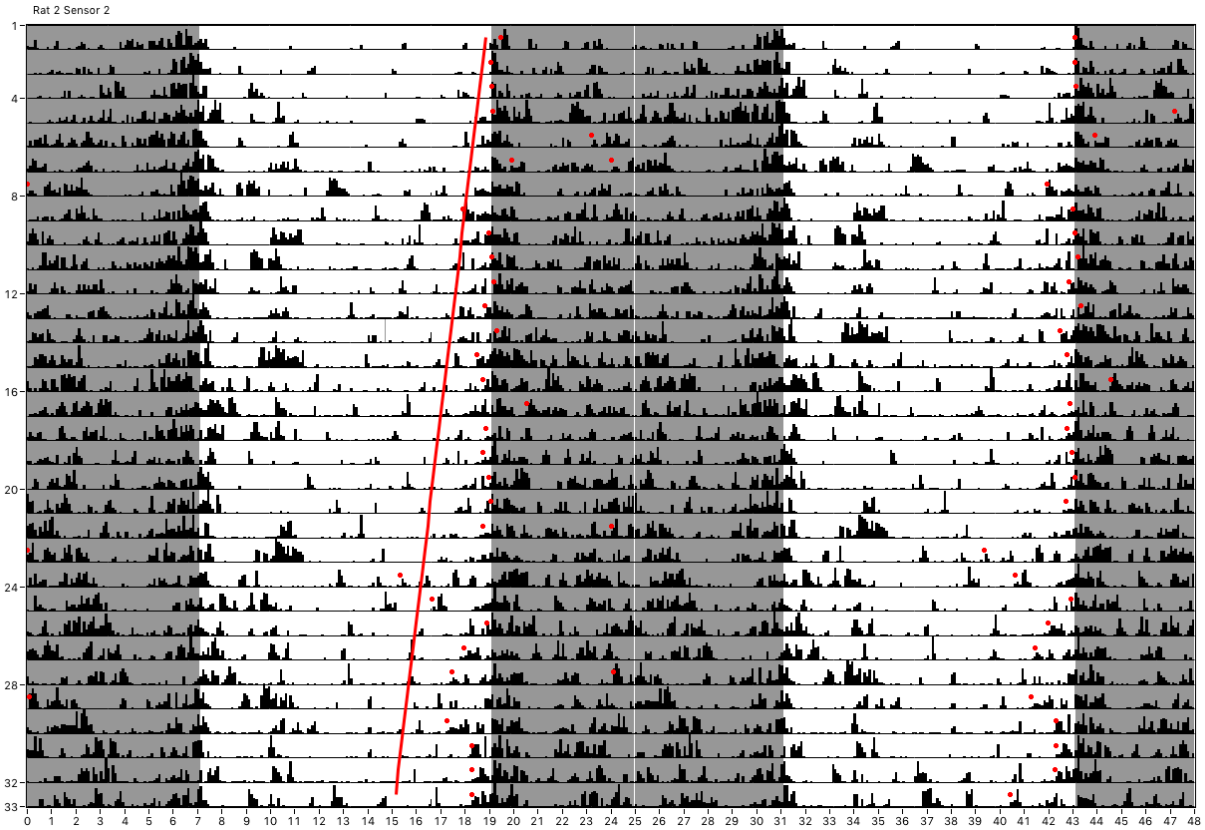
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The red line shows mean time onset of activity.

Figure 13. Actigram of Locomotor Activity in a DD-1M Rat Demonstrating FAA.



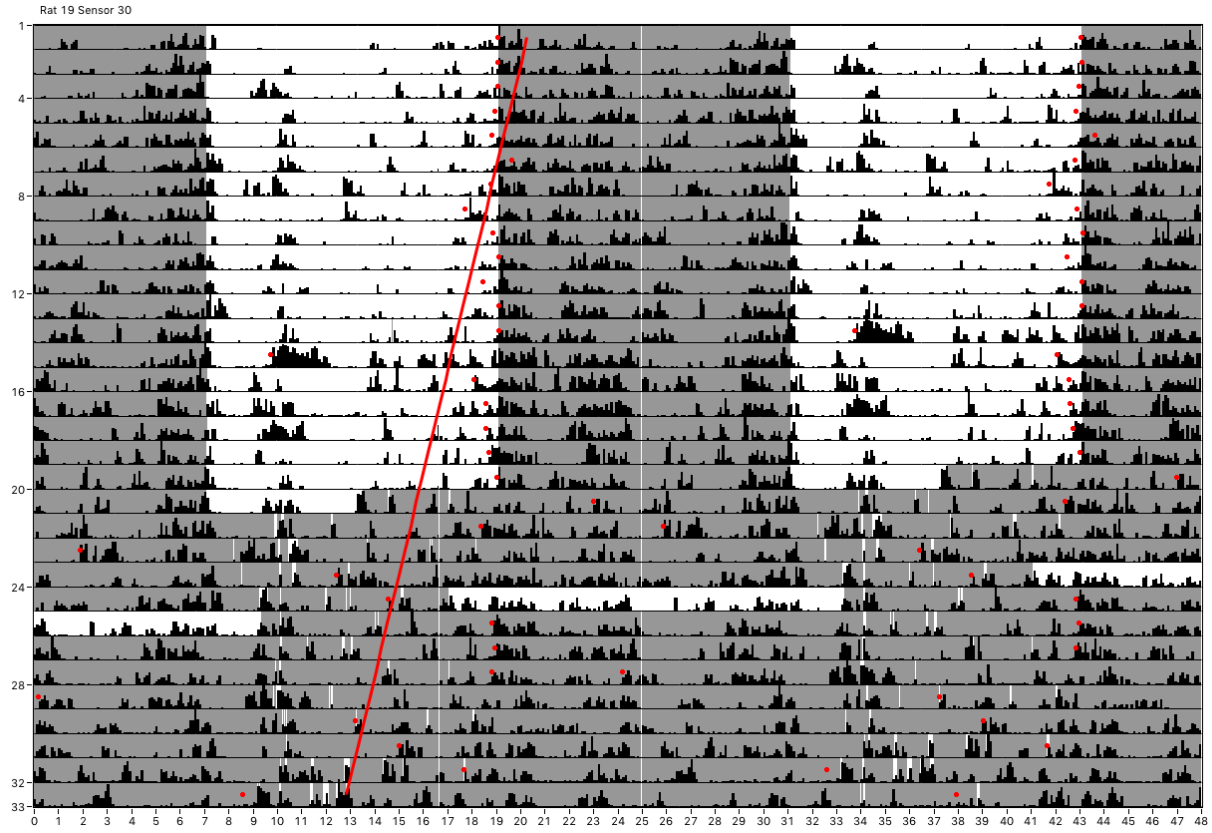
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). Abnormal white areas show when the red light was left on in the room, although this would not influence the rats. The red line shows mean time onset of activity.

Figure 14. Actigram of Locomotor Activity in a LD-AD Rat not Demonstrating FAA.



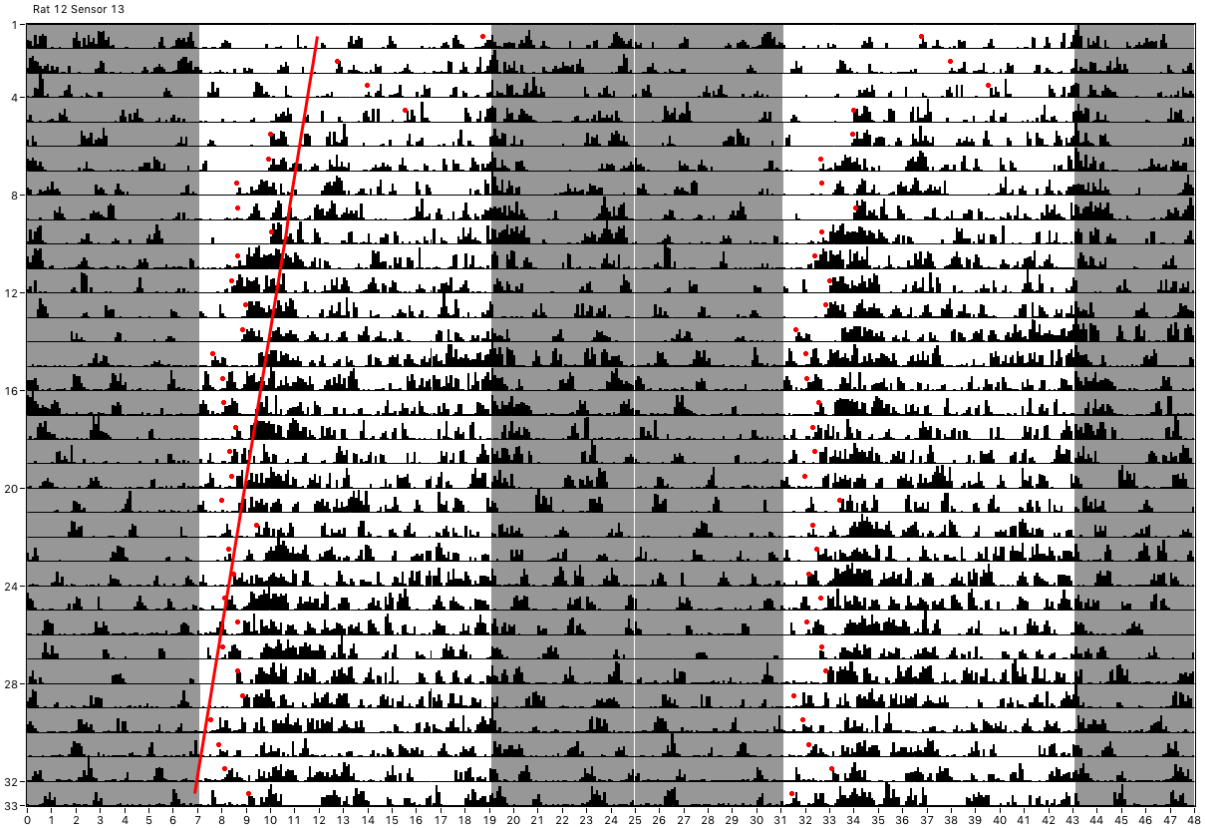
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The red line shows mean time onset of activity.

Figure 15. Actigram of Locomotor Activity in a DD-AD Rat not Demonstrating FAA.



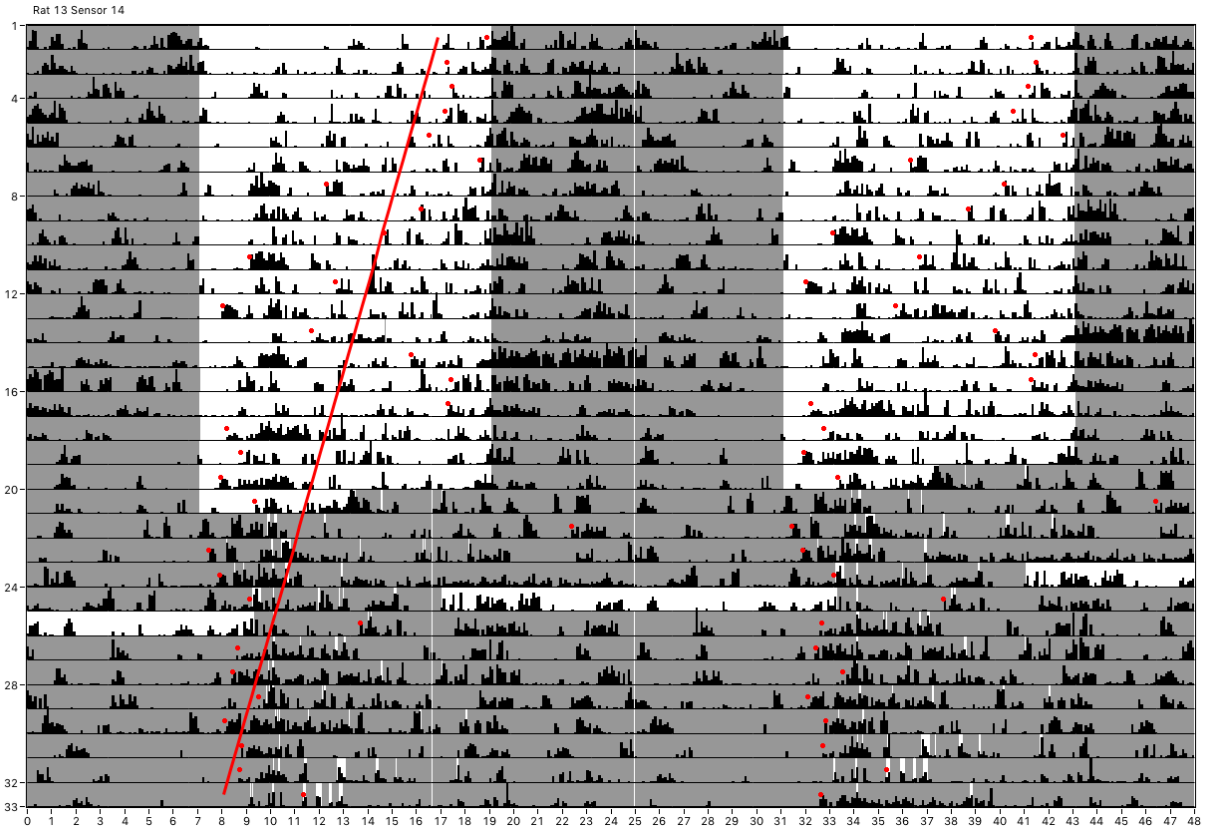
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). Abnormal white areas show when the red light was left on in the room, although this would not influence the rats. The red line shows mean time onset of activity.

Figure 16. Actigram of Locomotor Activity in a LD-MM Rat Demonstrating Small Amounts of FAA.



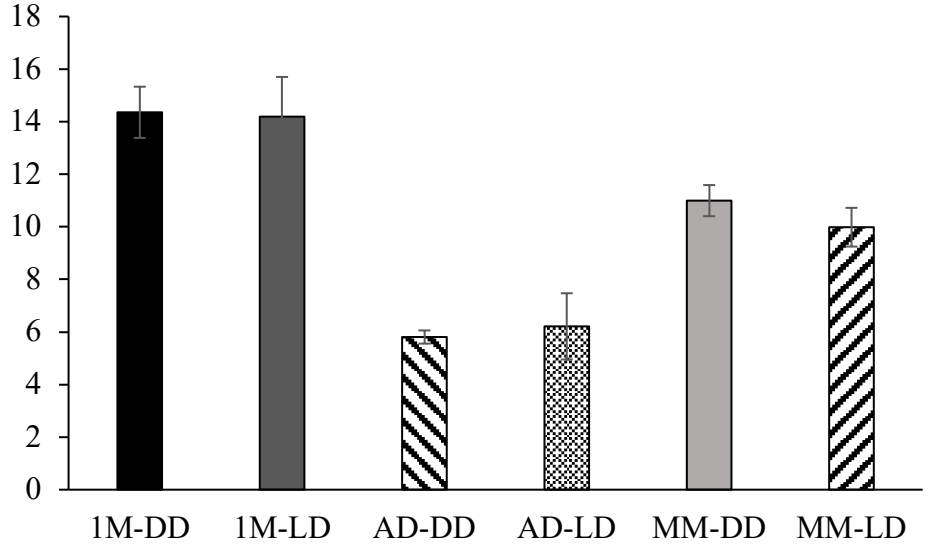
Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). The red line shows mean time onset of activity.

Figure 17. Actigram of Locomotor Activity in a DD-MM Rat not Demonstrating FAA.

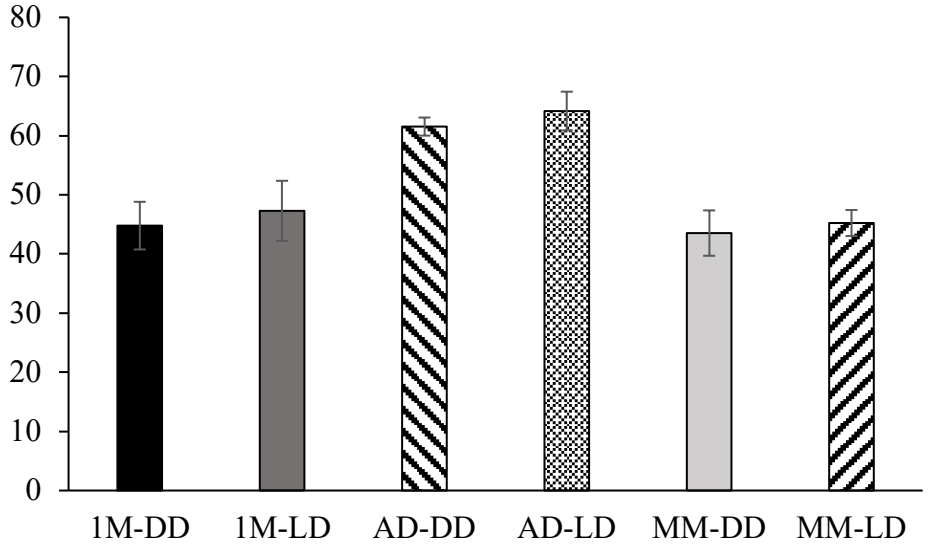


Note: Horizontal lines each show a full 24-hour period, with meal manipulations started on day 10. Gray areas represent the dark phase (lights off) while the white areas represent the light phase (lights on). Abnormal white areas show when the red light was left on in the room, although this would not influence the rats. The red line shows mean time onset of activity.

Figure 18. Clocklab Analyses: Study 3



A. Mean FAA Absolute Values with SEM. No differences between lighting groups. The MM group demonstrated significantly more FAA than the AD group. The 1M group showed significantly more FAA than the AD and MM group.



B. Mean Nocturnality Absolute Values with SEM. No differences between lighting group. The AD group showed significantly more dark-phase activity than the 1M and MM group.