CONTRIBUTIONS OF LIGHT- AND FOOD-ENTRAINABLE OSCILLATORS TO LEARNING DAILY TIME-PLACE TASKS

by © Anne-Marie Chaulk

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Abstract

Time-place learning (TPL) is the automatic encoding into memory information regarding the time and place of biologically significant events. Involvement of the suprachiasmatic nucleus (SCN) and the food-entrainable oscillator (FEO) in a daily TPL task was examined. Lesions eliminated the ability to use the SCN in SCNx rats, while unpredictable meal times prevented the use of the FEO in FEOx rats. Rats able to use either oscillator were expected to learn the task. Rats that could only use the SCN, or "master" circadian oscillator, were expected to perform better than rats that could only use the FEO. The ability to use both oscillators could enhance performance or impair learning due to suppression of one by the other. Impairment was expected for rats that could use neither oscillator. No differences were found between the groups, indicating that the use of neither oscillator may be necessary, and that there may be no benefit to having the ability to use either, or both. However, it is likely that unsuccessful lesions affected the results. Unexpectedly, FEOx rats preferred ordinal timing, contradicting previous findings. Replication of this study would be beneficial.

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Contributions of light- and food-entrainable oscillators to learning daily time-place tasks

The ability to automatically encode into memory information regarding the time and place of biologically significant events, referred to as time-place learning (TPL), is thought to be evolutionarily advantageous (Gallistel, 1990). When the location of a particular event changes reliably with the time of day, subsequent TPL is referred to as daily TPL (Thorpe, Deibel, Reddigan, & Fontaine, 2012).

Several species exhibit daily TPL. For example, bees learn which flower petals to land on in order to receive a reward when the reinforced petal varies with time of day (Gould, 1987). Likewise, garden warblers are able to learn which of four feeding rooms are reinforced at each of four times of day, and researchers showed that they accomplish this using a time-place map, as opposed to simply following a fixed route (Krebs & Biebach, 1989). Giant tropical ants (Harrison & Breed, 1987) and golden shiner fish (Reebs, 1996) successfully learn daily TPL tasks. Mice learn a connection between time and place when a reward-penalty paradigm is employed using a three-choice-arm maze (Van der Zee et al., 2008). The mice learned to visit safe baited arms and avoid arms on which they would receive a mild foot-shock (Van der Zee et al., 2008). Recently, social reinforcement was successfully used as a stimulus for TPL in zebrafish (Moura & Luchiari, 2016). Rats have also exhibited daily TPL in a number of experiments (Carr, Tan, & Wilkie, 1999; Carr & Wilkie, 1997b; Mistlberger, de Groot, Bossert, & Marchant, 1996; Pizzo & Crystal, 2002, 2004; Thorpe & Wilkie, 2007). For example, Carr and Wilkie (1997b) showed that rats in an operant box with a lever on each of the four walls learned to press one lever for a food reward in morning sessions, and a different lever for a food reward in afternoon sessions.

While several studies have examined daily TPL in animals, only a small subset has investigated the physiological mechanisms involved. In one of the first experiments on this topic, Mistlberger and colleagues (1996) studied the suprachiasmatic nucleus (SCN), as it is known to act as a circadian pacemaker in mammals (Dibner, Schibler, & Albrecht, 2010). Specifically, the SCN relies on input from the retina via the retinohypothalamic tract, that allows it to entrain to the light-dark cycle, making it a light-entrainable oscillator (LEO) (Dibner et al., 2010). Thus, it was hypothesized that the SCN could be involved in the solving of daily TPL tasks, acting as a clock (Mulder, Papantoniou, Gerkema, & Van Der Zee, 2014). For Mistlberger's (1996) experiment, male Wistar rats received bilateral lesions to the SCN prior to training on a T-maze task in which pressing the lever at the end of one of the choice arms in the morning resulted in a food reward, while pressing the lever at the end of the other choice arm resulted in a food reward in the afternoon. Rats with a lesioned SCN were not impaired on the task compared to control rats, indicating that the SCN was not necessary for learning a daily TPL task (Mistlberger et al., 1996). Instead, Mistlberger's team hypothesized that because the rats were always fed two meals per day at the same times of day, they could have used the reliable meal times to entrain a separate clock, the food-entrainable oscillator (FEO), and that this FEO could then allow them to learn the daily TPL task in the absence of the LEO (Mistlberger et al., 1996). However, they did not directly test whether the rats could still solve the task if they were not able to use the FEO.

Similarly, Boulos and Logothetis (1990) trained rats on a daily TPL task and manipulated their SCNs. One group had intact SCNs and were housed in constant light conditions, while another group had intact SCNs but were housed in a typical 12h:12h

light-dark cycle. The last group had SCN lesions and were kept in constant light. While the rats with intact SCNs that were subjected to a 12h:12h light-dark cycle performed best on the TPL task, the other groups learned as well. Like Mistlberger et al. (1996), they argued that because the rats with unreliable SCNs were fed two daily meals at the same times each day, they were able to use the FEO to perform the TPL task. However, they also did not manipulate access to the FEO. It is worth noting that performance was enhanced when the SCN was also available to the animals (Boulos & Logothetis, 1990). The purpose of the current study was to explore the interaction between the SCN and the FEO in learning a daily TPL task. In this study, along with manipulating the SCN, we explicitly manipulated the FEO, such that some rats had access to the FEO (i.e., by feeding them one meal per day at a consistent time of day) and other rats had no access to the FEO (i.e., by feeding them multiple meals per day at varying times of day).

Manipulating the number and timing of daily meals is an ideal way to manipulate the rats' access to the FEO, as previous research has shown that when rats are limited to one (Bolles & deLorge, 1962) or two (Boulos & Logothetis, 1990; Mistlberger et al., 1996; Mistlberger et al., 2012) meals per day, at the same time or times each day, they show food-anticipatory activity (FAA). FAA is an increase in activity preceding regularly scheduled daily feeding times, and signifies the operation of the FEO (Pendergast, Oda, Niswender, & Yamazaki, 2012; Pendergast & Yamazaki, 2014). The FEO may allow rats to solve daily TPL tasks when the SCN is lesioned as in the Mistlberger et al. (1996) and Boulos and Logothetis (1990) studies. Other research has also advanced the theory that the FEO is important in daily TPL (Reebs & Lague, 2000). Lukoyanov and colleagues (Lukoyanov, Pereira, Mesquita, & Andrade, 2002) used a Morris Water Maze task

(Morris, 1981) to show that rats fed one meal per day could learn to find the hidden escape platform which changed location based on time-of-day, while rats with *ad libitum* access to food were unsuccessful. This suggests that food entrainment is necessary to learn daily TPL tasks (Lukoyanov et al., 2002). However, data from subsequent studies conducted by Widman, Sermania, and Genismore (2004) indicated that the food restricted rats in the Lukoyanov et al. (2002) experiment were only able to learn the task due to the increase in response cost associated with the metabolic and energetic deficiencies induced by the severe food restriction. The highly food-restricted rats, as opposed to the rats provided with *ad libitum* access to food, would have had more motivation to find the hidden platform and escape the water, as doing so would prevent subsequent expenditure of valuable depleted energy stores. Widman's team (2004) conducted two experiments using the Morris Water Maze (Morris, 1981), the first of which replicated the findings from the Lukoyanov et al. (2002) experiment and indicated that rats could not learn the time-place discrimination when provided with *ad libitum* access to food. For the second experiment, weighted belts were placed on rats to increase response cost, and they were then able to successfully learn the daily TPL task, despite having been provided with *ad libitum* access to food. This supports the theory that the food-restricted rats in the Lukoyanov et al. (2002) experiment were successful because of the increased response cost, and not because of access to the FEO. For the current TPL experiment, we equated the response cost between groups by placing them all on restricted feeding schedules. To vary access to the FEO, we followed the procedure previously implemented by researchers in our lab (Wall et al., 2019). Rats were either fed once per day at the same time each day, allowing them to use the FEO, or at multiple semi-random times per day,

preventing use of the FEO. Multiple semi-random feedings were used as a method of eliminating the potential for FEO use, as opposed to simply providing food *ad libitum*. This was so that the rats would remain motivated to perform the experimental task, for which food was the reward for successful completion, and because if some rats were given *ad libitum* food, those rats would have a lower response cost than would the rats on a restricted feeding schedule.

The goal of the current study was to better understand the role of both the LEO and the FEO in the acquisition of daily TPL by manipulating both within the same study. Mistlberger et al. (1996) lesioned the SCN, the known site of the LEO, but did not directly manipulate the FEO. Lukoyanov et al. (2002) attempted to manipulate the FEO, but inadvertently confounded response cost by feeding the FEO rats substantially less food. We attempted to study the interaction between the LEO and FEO by lesioning the SCN in some of our animals and varying the reliability of meal times to manipulate the FEO. While it is possible to lesion the site of the LEO (i.e., the SCN), this is not possible for the FEO as the exact anatomical locus of the FEO remains unknown (Munn, Tyree, McNaughton, & Bilkey, 2015). It is known that it does not reside in the SCN because when the SCN is lesioned, FAA is unaffected and still present, indicating that alternative brain regions are involved in the FEO (Stephan, 2002). Multiple structures in the brain likely form a network to produce the FEO (Carneiro & Araujo, 2009; Escobar, Cailotto, Angeles-Castellanos, Delgado, & Buijs, 2009; Mulder et al., 2014), of which the hippocampus may be a part (Munn & Bilkey, 2012; Munn et al., 2015). Humoral pathways involving hormones have been implicated in food intake regulation and may be involved in the FEO (Carneiro & Araujo, 2009). Possible loci have been suggested and

subsequently discarded, such as the digestive system (Davidson, Poole, Yamazaki, & Menaker, 2003) and the liver (Damiola et al., 2000; Davidson, Stokkan, Yamazaki, & Menaker, 2002; Stokkan, Yamazaki, Tei, Sakaki, & Menaker, 2001). Because the site of the FEO is unknown, we relied on environmental manipulations such as varying the regularity of meal times.

The current study was designed to examine the role the LEO and FEO play in the *acquisition* of daily TPL. Most previous researchers have attempted to uncover the role of these oscillators in daily TPL by manipulating them in rodents that have already mastered the TPL task (e.g., Mulder et al., 2014, Experiment 1). Several studies involving the use of the SCN or the FEO have been conducted in an attempt to discern the differential reliance on each of these systems (Angeles-Castellanos, Salgado-Delgado, Rodriguez, Buijs, & Escobar, 2010; Blum, Waddington Lamont, & Abizaid, 2012; Boulos & Logothetis, 1990; Bradley & Prendergast, 2014; Mulder et al., 2014; Reebs & Lague, 2000). It appears mice tend to use both the SCN and FEO during a TPL task, unless they are unable to do so, in which case, whichever oscillator is still dependable may come to be relied upon alone (Mulder et al., 2014). In an experiment that assessed the roles of the LEO and the FEO in daily TPL, mice were trained on a three-session-per-day aversive TPL task (Mulder et al., 2014). Mice had to learn to avoid a mild foot-shock at one of three baited locations based on the time of day. In a correct session, the mouse either did not visit the shocked location, or visited the other two locations first. Intense light pulses occurring at the beginning of the dark phase were used to phase delay the SCN-dependent circadian rhythms in mice that had successfully learned the daily TPL task. The performance of these mice in all sessions suffered as a result of the manipulation, with the

effect persisting for two to three days. Once performance returned to previous levels, rats were fed six hours later than normal, affecting the circadian rhythms associated with the FEO. This manipulation resulted in a one day drop in performance levels in certain sessions. Next, a six-hour food advance resulted in poorer performance in certain sessions, lasting for two days. It is clear from the decreases in performance following the phase shifting and meal time adjustments that TPL is affected by alterations to the LEO and FEO, but because performance recovered quickly, it is likely that TPL does not fully rely on the normal operation of either oscillator. In a second experiment (Mulder et al., 2014), one group of mice received SCN lesions and another group received sham surgeries. All mice were subsequently trained on the TPL task. Performance did not differ between groups, indicating that the SCN is not essential for the acquisition of TPL. As task performance can be affected by alterations to both the LEO and FEO, it is proposed that a network of brain regions, that encompasses the LEO (SCN), FEO, and other areas involved in memory processes, acts as an internal clock that can be consulted to aid in TPL (Mulder et al., 2014).

If multiple systems have evolved to entrain animals to the 24-hour day, there would likely be interactions between these systems. For example, it is possible that these systems emerged so that if one oscillator was unavailable or unreliable, another oscillator could compensate. There is evidence from areas outside of the TPL literature that suggests these oscillators do in fact interact with one another. For example, a link between the SCN and the FEO was suggested in a study conducted by Reebs and Lague (2000). Golden shiners were maintained on a 12h:12h light-dark cycle and fed once daily at the same time each day. FAA was observed in these fish. When the fish were

subsequently placed in constant darkness, the majority stopped exhibiting FAA. This could indicate that the FEO is linked to an LEO, and that if the LEO cannot function properly, there is a detrimental effect on the operation of the FEO (Reebs & Lague, 2000). Similarly, Bradley and Prendergast (2014) found that the strength and persistence of FAA in Siberian hamsters can be influenced by the light-dark cycle. Hamsters exposed to a short day (nine hours of light), as opposed to a long day (15 hours of light), showed higher and more persistent levels of FAA preceding daily timed access to food. The dorsomedial nucleus of the hypothalamus communicates with the SCN and inhibits the SCN's influence over circadian rhythms when food is scarce, thus allowing the FEO to exert control (Blum et al., 2012). Finally, there is some research to suggest that the outputs of the SCN may have to be suppressed in order for the outputs of the FEO to be expressed (Angeles-Castellanos et al., 2010; Blum et al., 2012). When rats with SCN lesions were compared to rats with intact SCNs, they showed an earlier onset and a greater degree of FAA (Angeles-Castellanos et al., 2010). Previous investigations into the use of the SCN and the FEO for daily TPL tasks have not led to a clear understanding of the roles of each oscillator, nor have they clarified the possible interactions between them. With this study, we attempted to fill these gaps in the literature.

Another question that we aimed to answer with this study was whether the oscillators used would influence the type of timing strategy employed by the rats. Previous work has shown that, depending on the specifics of the experiment, rats can use either an ordinal, interval, circadian, or alternation strategy to solve daily TPL tasks (Carr & Wilkie, 1997b; Deibel & Thorpe, 2013; Pizzo & Crystal, 2002, 2004). To determine which timing strategy rats were using, skip session probes were conducted after the rats

mastered the TPL task. A rat undergoing a skip session probe is left undisturbed in the colony room at the time of one of the two daily experimental trials. If rats are using a circadian strategy, then they have learned the time of day associated with each place. Following skipped sessions, rats using a circadian strategy will continue to successfully solve the task (e.g., Deibel & Thorpe, 2013). If rats are using an ordinal strategy, then they have learned the order in which the locations provide food within a given day. Following skipped morning sessions, rats using an ordinal strategy will incorrectly go to the morning location even though it is now the afternoon. However, following skipped afternoon sessions, they will go to the correct location the next morning (e.g., Carr et al., 1999; Carr & Wilkie, 1997b). Occasionally rats have also been found to use an alternation (i.e., non-timing strategy in which rats learn to avoid the most recently reinforced location), interval (e.g., food will be in Location A 5 hours after the colony room lights come on), or a combination of strategies (e.g., Deibel & Thorpe, 2013; Pizzo & Crystal, 2002, 2006). While evidence has been found for all of these strategies, it is unknown if the oscillator employed (FEO vs. LEO) influences the strategy used.

To summarize, the purpose of the current study was to determine the roles of the LEO and the FEO in the acquisition of a daily TPL task. We manipulated whether the rats had access to the LEO by lesioning the SCN in some of the rats. We manipulated whether rats had access to the FEO by varying the number and timing of meals the rats had per day. Importantly, all rats were food restricted so that there were no differences between groups in response cost and motivation. Finally, we conducted skip session probes to determine if there was a relationship between the oscillator being used and the timing strategy employed. It was hypothesized that rats with access to either the SCN or the FEO

would be able to learn the task. It was unknown whether rats with access to both the SCN and the FEO would learn faster or slower than those with only one of the two available. The oscillators could be in competition with one another and the SCN could suppress the ability of the FEO to contribute any additional assistance or, because food was scarce, the influence of the SCN could be inhibited, allowing the FEO to exert control (Angeles-Castellanos et al., 2010; Blum et al., 2012). Alternatively, the SCN and FEO could work together to enhance performance (Boulos & Logothetis, 1990). Those rats that had access to only the SCN were expected to perform better than those with only the FEO available, as the SCN is the "master" circadian oscillator (Blum et al., 2012; Dibner et al., 2010). Based on previous research, we felt confident that rats that had access to neither the FEO nor the SCN would be impaired in learning the task.

Circadian rhythm disruption has been implicated in human disorders that affect memory, such as Alzheimer's disease (Harper et al., 2005). Animal studies that elucidate the relative roles of the SCN and the FEO in memory functioning have implications for human research, and the possible interactions between the oscillators could prove important in advancing treatment options for those suffering from memory disfunction.

Method

Subjects

Fifty-two male Long-Evans rats obtained from Charles River (St. Constant, QC, CA) were housed individually in conventional plastic cages (45 cm x 25 cm x 21 cm) with metal covers and corncob bedding (Necto Company, New York, NY, USA). Shredded paper (Crink-l'Nest, Kraft, The Andersons, Inc., Maumee, OH, USA), wooden blocks, Nylabones (Nylabone Products, Neptune, NJ, USA), cotton squares, and hard

plastic hollow tubes were provided to all rats in their home cages. Rats were housed in these cages until at least a week post-surgery. At this time, they were transferred from their home cages to individual specialized cages, each of which was attached to a running wheel. Each running wheel cage was made of clear Plexiglas measuring 39.5 cm x 17.5 cm x 17.5 cm and had a removable cover that could be locked into place. Sawdust (P.W.I. Industries Inc., St-Hyacinthe, QC, CA) covered the floor. On one side of the cage, the rat had free access through a 12.5 cm x 10 cm rectangular hole, to a metal running wheel (12 cm wide and 36 cm in diameter). Running wheel data were collected and saved in one second bins and then transferred to the statistical program R, for which a program had been written that was used to create actograms depicting a visual representation of the rats' activity levels over time. Actograms allow for the determination of the time of peaks of activity.

The light-dark cycle was kept constant at 12h:12h, with lights on at 07:00 and off at 19:00. Water was provided *ad libitum*, except during lever press training and experimental trials. Laboratory Animal Feed (PMI Nutrition International, St. Louis, MO, USA) was available *ad libitum* for the first week, after which rats were placed on restricted feeding schedules. Rats were permitted to gain 10 g per week. Some of the rats were fed one meal per day at 16:30, and therefore had access to the FEO. The rest were each fed one to three smaller meals at semi-random times throughout the day which prevented use of the FEO. All feedings took place during the light phase and were separated by at least one and a half hours. Rats that were included in the analyses weighed between 260 g and 442 g at the start of experimental trials, with an average of 347 g and were between 86 days and 197 days old, with an average of 133 days.

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

All rats were individually trained to lever press in a Plexiglas operant box (47 cm x 47 cm x 32 cm), that had 2 cm of sawdust (P.W.I. Industries Inc., St-Hyacinthe, QC, CA) covering the floor and was placed on a table in a room measuring 243 cm x 182 cm. Each wall of the box contained: a retractable lever (Med Associates Inc., St. Alban, VT, USA, model number ENV-112CM), a food cup (Med Associates Inc., St. Alban, VT, USA, model number ENV-200R1AM), and a pellet dispenser (Med Associates Inc., St. Alban, VT, USA, model number ENV-203-45). A small light near the middle of each wall was illuminated when the corresponding lever was activated. The reinforcement for lever pressing was a 45 mg food pellet (Dustless Precision Pellets, BioServ, Frenchtown, NJ, USA).

Experimental trials were conducted on an open T-maze, raised 85 cm off the floor, with the start arm and choice arms measuring 15 cm x 53 cm each. At the end of each choice arm, a clear Plexiglas wall (28 cm x 15 cm) contained a non-retractable lever (Med Associates Inc., St. Alban, VT, USA, model number ENV-110M), a food cup (Med Associates Inc., St. Alban, VT, USA, model number ENV-200R1AM), and a pellet dispenser (Med Associates Inc., St. Alban, VT, USA, model number ENV-203-45). A small light near the middle of each wall was illuminated for the duration of the session. The T-maze was located in a room measuring 6 m x 2.5 m that contained several fixed objects, such as: an air conditioner, a desk with a radio and controller box, a counter,

posters, a wooden chair, and a purple curtain that hung almost from the ceiling to the floor. Rats were placed on a cart behind the curtain during the experimental trials. A radio played during all training sessions.

Surgery

Rats at the time of surgery were between 65 days and 106 days old, with an average of 76.5 days. Rats received either bilateral electrolytic lesions to the SCN or a sham surgery. Sham surgeries involved the same procedure as lesion surgeries, however the current of the electrode was not turned on and, therefore, the electrode did not damage the SCN. Due to time constraints, for 73% of the sham surgeries, the electrode was not lowered but the rest of the procedure remained the same.

Prior to surgery, rats were anesthetized with a mixture of isoflurane and oxygen. The rats were placed in a stereotaxic instrument and small holes were drilled bilaterally into the skull above the location of the SCN. For one of the sham surgeries, no holes were drilled in the skull due to equipment malfunction. An electrode was lowered on an 8˚ angle to the coordinates for the SCN: AP: -0.8, ML: ±1.4, DV: -9.0. Bregma was used as the reference point for these coordinates. Coordinates were altered slightly for some rats, based on weight at the time of surgery. A 0.2 mA current was passed through the electrode for five seconds to lesion the SCN. The same method was used for both sides.

Procedure

Upon arrival, rats were given one week to habituate to their new environment and human handling, during which time they were handled daily. They were then placed on restricted feeding schedules and permitted to gain 10 g per week. The restricted feeding schedules allowed access to the FEO to be established in the appropriate groups prior to

surgery. Rats in Group SCN / FEO (n = 10) underwent sham surgery which resulted in no damage to the SCN, and therefore caused no disruption to the normal functioning of the SCN. They were fed one meal per day at 16:30 and therefore also had access to the FEO. Rats in Group SCN / FEOx (n = 9) also underwent sham surgery which caused no damage to the SCN. These rats were each fed one to three smaller meals per day at semi-random times and therefore could not use the FEO. Rats in Group SCNx / FEO (n = 17) received bilateral electrolytic lesions to the SCN and were fed once per day at 16:30, allowing them access to the FEO. Rats in Group SCNx / FEOx (n = 16) also received bilateral electrolytic lesions to the SCN but were each fed one to three smaller meals per day at semi-random times and therefore could not use the FEO. See Table 1 for a summary of which oscillators were available to rats in each group.

Following recovery from surgery, all rats were transferred to individual cages attached to running wheels to which they had free access for approximately 21 days prior to the start of training. Wheel running activity was monitored to allow confirmation that the rats with SCN lesions were not entrained to the light-dark cycle (Cain, Chalmers, & Ralph, 2012; Cain, Featherstone, & Ralph, 2011). Actograms depicting activity levels were created using this data. If peaks of activity showed no clear pattern (i.e., arrhythmia), the circadian rhythm was deemed to be no longer entrained to the light-dark cycle. The extent of FAA could also be determined from the actograms (Mistlberger, 1994). The presence of peaks of activity immediately preceding feeding times is indicative of FAA, and the size of these peaks represents the degree of FAA present.

Once rats were removed from the running wheel cages and returned to their home cages, they were trained to lever press on a variable ratio (VR) 15 schedule, meaning they

were reinforced after an average of 15 presses. They were then trained on the experimental task. Data collected from rats that were 200 days or older at the start of the experiment, due to such factors as prolonged recovery from surgery or difficulty with lever press shaping, were not included in the analyses. See Table 2 for a complete listing of rats that were excluded. Four-minute experimental sessions were conducted for each rat, one in the morning and another, six hours later, in the afternoon. Rats were required to go to different baited locations on a T-maze, depending on the time of day, to receive a food reward (pellet). Only one location was reinforced at a time. The locations of the reinforced levers were counterbalanced amongst the rats and all reinforcement followed a VR 5 schedule. All lever press training and experimental trials were conducted during the light phase of the light-dark cycle.

Rats were said to have learned the task when they reached a previously determined criterion of 18 correct first lever presses out of 20. Rats that did not reach criterion by Day 80 of the experimental trials were not expected to learn and were therefore removed from the experiment. Once the rats had learned the task, skip session probes were conducted to determine the type of timing strategy employed. For an individual rat, two morning skips and two afternoon skips were conducted. If the results of the two morning skip sessions did not agree, a third skip session was used as a tie-breaker. This tie-breaker method was also employed in the afternoon if the two afternoon skip sessions yielded differing results. Between skip sessions, rats had to reach a criterion of four out of five correct trials or four correct trials in a row. When a skip session probe was conducted, the rat was not brought into the experimental room as usual, but was left undisturbed in the colony room.

Histology

After the rats completed the experimental task and all skip session probes, they were sacrificed using a gas mixture of carbon dioxide and oxygen. Immediately after, they were decapitated, and the brains were extracted and frozen in a container of 2-methylbutane**.** See Figure 1 for a timeline showing the complete procedure for the rats**.** A cryostat microtome (Leica CM3050 S) was used to take several 30 micrometre coronal sections of the brains from the area in which the SCN is normally found. Sections were mounted on glass slides that had been coated with a chrom alum and gelatin solution. All sections were stained with cresyl violet, cover-slipped, and allowed to dry. Sections were examined using a microscope (Bausch & Lomb) to determine the extent of any lesions present. Figure 2 shows an image of a brain section from: a) a complete, b) a partial, and c) a sham lesion. One rat from Group SCNx / FEO was excluded from the analyses, as problems with sectioning prevented verification of a successful lesion.

Results

Our initial analyses included the 40 rats that are shown in Table 2. The final number of rats in each group was as follows: Group SCN / FEO (*n*=8), Group SCN / FEOx (*n*=7), Group SCNx / FEO (*n*=12), and Group SCNx / FEOx (*n*=13). While it is more typical to first determine whether the lesions were successful prior to doing any statistical analyses, we chose to do the statistical analyses with all rats because we were interested in not only the comparison between the lesion and sham groups but also in the comparison of the groups with and without access to the FEO.

To determine if there were any differences between groups in acquisition of the TPL task, we calculated the number of days to criterion. Criterion was defined as 18/20

correct first presses. If a rat did not reach criterion before Day 80 of experimental trials, a "best case scenario" for days to criterion was calculated by assuming the rat would have performed all following sessions correctly if training were to have continued. The mean days to criterion for each group are shown in Figure 3. An analysis of variance (ANOVA) comparing the average days to criterion for each group indicated there were no significant differences between the groups, $F(3, 36) = 0.385$, $p = 0.764$, partial $\eta^2 = 0.031$. There was no main effect of lesion, $F(1, 36) = 0.512$, $p = 0.479$, partial $\eta^2 = 0.014$, and no main effect of meal group, $F(1, 36) = 0.492$, $p = 0.488$, partial $\eta^2 = 0.013$. There was no lesion x meal group interaction, $F(1, 36) = 0.016$, $p = 0.901$, partial $\eta^2 < 0.0001$. Another ANOVA was conducted in the same manner to check for significant differences between the groups with respect to days to criterion, but with criterion set at 16 correct trials out of 20. Best case scenarios for rats that did not reach criterion were computed in the same way as they were for the first ANOVA. Once again, there were no significant differences between the groups, $F(3, 36) = 0.064$, $p = 0.978$, partial $\eta^2 = 0.005$. There was no main effect of lesion, $F(1, 36) = 0.025$, $p = 0.875$, partial $\eta^2 = 0.001$, and no main effect of meal group, $F(1, 36) = 0.141$, $p = 0.709$, partial $\eta^2 = 0.004$. There was no lesion x meal group interaction, $F(1, 36) = 0.052$, $p = 0.821$, partial $\eta^2 = 0.001$.

Given that there were no significant differences between groups, we categorized rats that underwent lesion surgery as either a complete, a partial, or a miss, based on the accuracy and extensiveness of the SCN lesion (see Figure 2 for an example of a complete, a partial, and a sham lesion). From Group SCNx / FEO, three rats had complete lesions, eight had partial lesions, and one was a miss. The lesion of one of the rats from Group SCNx / FEO could not be confirmed due to problems with brain sections that prevented

histology from being completed. From Group SCNx / FEOx, two had complete lesions, six had partial lesions, and five were misses. Data from one rat from Group SCNx / FEOx were excluded from analyses due to a computer glitch which erroneously made it appear that criterion had been reached, resulting in a premature probe.

Table 3 shows the days to criterion for each rat, as well as whether the lesion was "complete", "partial", or a "miss", whether the rat showed rhythmicity, and the timing strategy employed for the task. Unfortunately, with such small samples of complete lesions in the two lesion groups, meaningful statistical analyses could not be completed on days to criterion for only those rats with complete lesions.

Next, we examined the actograms to determine if some of those rats with partial lesions were arrhythmic and could therefore be used in the analyses. Actograms depicting the running wheel data were created and analyzed to determine whether each rat had rhythmic or arrhythmic activity. The actogram of a rat with an intact SCN should indicate rhythmic activity, while that of a rat with a lesioned SCN should indicate arrhythmic activity. While partial SCN lesions can impact circadian rhythms, they do not have the effect of immediately and completely eliminating rhythmicity (Eastman, Mistlberger, & Rechtschaffen, 1984). As subjective visual examination was employed, several actograms were not clearly rhythmic or arrhythmic, and when this was the case, the majority opinion of five researchers in our lab was used to make a final decision. The researchers were blind to the group to which the particular actogram belonged before classifying it as rhythmic or arrhythmic. FAA appeared to be present for some of the FEO rats but was less clear for others. Figures 4a, 4b, 4c, and 4d show the clearest actogram from each group, respectively. Based on the actograms, as well as histology, it would appear that

only two rats were successfully lesioned in each group, which is not enough for analyses. Even with the inclusion of the third rat in Group SCNx / FEO that was deemed to have a complete lesion following histology, there were not enough rats with successful lesions to make analyses possible. Several of the actograms indicated that rats tended to be most active in the light phase of the light-dark cycle. Gritton, Kantorowski, Sarter and Lee (2012) found that, while rats are normally nocturnal, a switch to diurnality can occur when rats are trained on an attentionally demanding task in the light phase. Perhaps the task in the present study was demanding enough to encourage such a switch.

Skip session probes were used to determine which timing strategy was employed by those rats in each group that learned the task (i.e., reached criterion, see Table 3). Of the six rats that learned the task and completed all skip session probes in Group SCN / FEO, five used a circadian strategy. For the remaining rat, a strategy could not be determined because the results of the skip session probes did not correspond with either a circadian or ordinal strategy. Of the seven rats that learned the task and completed all skip session probes in Group SCN / FEOx, three used a circadian strategy and four used an ordinal strategy. Of the nine rats that learned the task and completed all skip session probes in Group SCNx / FEO, six used a circadian strategy and three used an ordinal strategy. Of the ten rats that learned the task and completed all skip session probes in Group SCNx / FEOx, three used a circadian strategy and six used an ordinal strategy. For the remaining rat, a strategy could not be determined because the results of the skip session probes did not correspond with either a circadian or ordinal strategy.

Discussion

The purpose of the current study was to determine the roles of the LEO and the FEO in the acquisition of a daily TPL task. We manipulated whether rats had access to the FEO by altering the number of meals the rats had per day. We also ensured that all rats were food restricted so that there were no differences between groups in response cost and motivation. Unfortunately, our attempts to manipulate the LEO were not as successful. To manipulate access to the LEO we lesioned the SCN in some of the rats. However, we only successfully destroyed the entire SCN in five rats. While there were an additional 14 rats that had partial lesions of the SCN, previous research has shown that rats can maintain or regain rhythmicity if a portion of the SCN remains (Eastman et al., 1984; Ohtsuka-Isoya, Hayashi, & Shinoda, 2001). Ohtsuka-Isoya et al. (2001) studied the effect of SCN lesions on the circadian rhythmicity of the periodic incremental lines that occur in the dentin of rats' teeth. While complete lesions abolished rhythmicity, partial lesions only temporally disrupted rhythmicity or did not disrupt it at all. In those rats in the current study with a partial lesion, we only saw corresponding arrhythmicity in seven rats. Unfortunately, because of these small sample sizes, we were unable to determine the effect of SCN lesions on the acquisition of the daily TPL task. This further meant that we could not determine how the LEO and FEO interact in the acquisition of daily TPL tasks.

Studying the effects of SCN lesions on acquisition of daily TPL is a challenging task. First, the SCN is a relatively small structure (Liu, Zhang, Xu, Huang, & Qu, 2012; Ohtsuka-Isoya et al., 2001) located in the hypothalamus. In order to lesion the SCN, an electrode must be lowered at an 8˚ angle to a depth of DV: -9.0. The small size of the SCN, as well as the angle and the fact that it is so deep in the brain, makes it a difficult

structure to lesion completely. Second, if even a portion of the SCN remains, rhythmicity may be preserved or only temporarily affected (Eastman et al., 1984; Ohtsuka-Isoya et al., 2001). Third, we were unable to confirm the lesions until months after the surgeries. After recovery from the surgery, rats started training on the task. Many of the rats were trained for 80 days of experimental trials before they were removed from the daily TPL task. The experiment was normally run 5 to 7 days per week, meaning that some of the rats were training for several months. For those that did learn the task, they also had skip session probes to confirm the timing strategy used. These skip session probes typically took at least 4 to 6 weeks to complete. Therefore, a significant investment of time and effort was expended on each rat and it took approximately 7 months post-surgery to discover that the majority of the lesions in a given cohort were unsuccessful. At this point adjustments were made to coordinates, but again months passed before it could be determined whether these lesions were successful. Prior to the start of the study we did do a number of pilot studies, however given that reported successful lesion rates in published literature are as low as six percent (Liu, et al., 2012), our success rates are not that surprising. We attempted to determine whether rats were arrhythmic prior to starting training on the daily TPL task, however this was not completely possible for two reasons. First, for lesioned rats that were fed one daily meal, seeing rhythmic patterns of behaviours may have been due to the use of the FEO. Therefore, it was only in the lesioned rats fed multiple meals per day that this strategy would be useful. Second, for all of the rats, even the non-lesioned ones, there was considerable variability in activity. Running wheel revolutions are not the only way that circadian rhythms can be represented. Gritton, Stasiak, Sarter, and Lee (2013) found that body temperature, tracked by intra-abdominal transmitters, was

a more reliable way to represent the output of the SCN. This method could prove more reliable and could be considered for future replication studies.

Unfortunately, we also did not see an advantage for rats that had access to the FEO. This is surprising given that previous research in our lab (Wall et al., 2019) found that rats fed one meal per day in an operant box version of the daily TPL task learned the task quicker than rats that were fed multiple meals per day. While the TPL paradigm was similar to that in the present study, there were differences that may have contributed to the contradictory findings. It is possible that the use of the FEO is somehow more advantageous for rats in an operant box than it is for those on a T-maze. Another difference between the studies was the differential use of timing strategies. Of the FEO rats for which a timing strategy could be determined, the majority in both studies used a circadian strategy as opposed to an ordinal strategy (Wall et al., 2019). There was a difference between the FEOx rats, with those in the current study tending to be ordinal timers, and those in the previous study tending to be circadian timers (Wall et al., 2019). The most obvious difference in procedures was the fact that rats in the present study underwent anesthesia, surgery, and recovery, any of which could have had lasting effects which affected performance. The only rats in the current study that preferred an ordinal rather than circadian timing strategy were the rats that did not have access to an FEO. Perhaps the preferred strategy in SCN-intact rats is circadian (as seen in Wall et al., 2019) but the surgeries in the current study, even in sham rats, impacted the SCN or some other part of the circadian system. Without access to the compensating efforts of an FEO, the FEOx rats resorted to using an ordinal strategy. Alternatively, the sample size in the current study was larger than that in the Wall et al. (2019) study and it is possible that,

with a larger sample size, Wall may have seen results more consistent with the present findings. It is important to note that, if it is the case that the surgeries even in the sham rats impacted the circadian system in some way, it could contribute to the reason for our lack of differences between experimental groups. Perhaps alternative methods for disrupting the functioning of the SCN should be considered in the future.

Fortunately, lesions are not the only way to manipulate the SCN. Future studies could use lighting manipulations to disrupt functioning. Rats in other studies have been exposed to photoperiod shifts (McDonald et al., 2013) or constant light (Eastman & Rechtschaffen, 1983) as a method of disrupting the SCN and circadian rhythmicity. Evidently, these are easier techniques than performing lesion surgeries to disrupt the functioning of the SCN. There are detriments to these alternative methods however, such as prolonged stress that could affect the results of any subsequent experiments. The arrhythmicity accomplished through lighting manipulations is also temporary, as opposed to the permanence of a lesion. This necessitates either a prolonged exposure to photoperiod shifts or a relatively quick running of experimental trials, which is not usually possible for TPL tasks that can take weeks or months of daily training to complete. The firing rate of neurons in the SCNs of mice can be manipulated using optogenetics (Jones, Tackenberg, & McMahon, 2015). This technique results in lasting changes and should be explored further as a possible method for manipulation of the rat SCN in TPL studies, as it would negate the problems associated with surgery, such as partial lesions and misses, hopefully leading to amplified differences between groups.

The study of mammalian circadian oscillators and their importance for TPL is an exciting area of research and should continue despite disappointing outcomes. There is

much to be learned regarding the circumstances under which one oscillator prevails over the other, and when they might work together, as well as what permits or inhibits such cooperation. Such research could have implications, not only for further animal studies, but for human studies as well. Perhaps learning more about oscillators and their roles in memory might lead to the discovery and development of new treatments for disorders that involve a deficit in memory function, specifically those disorders, such as Alzheimer's disease, that are impacted by a disruption in circadian rhythm (Harper et al., 2005). Despite the lack of significant results in the current study, the design is valid and the information we hoped to gather is important. Experimental techniques can be improved upon and further studies conducted in the future.

References

Angeles-Castellanos, M., Salgado-Delgado, R., Rodriguez, K., Buijs, R. M., & Escobar, C. (2010). The suprachiasmatic nucleus participates in food entrainment: A lesion study. *Neuroscience, 165*, 1115-1126. doi:10.1016/j.neuroscience.2009.11.061

Blum, I. D., Waddington Lamont, E., & Abizaid, A. (2012). Competing clocks: Metabolic status moderates signals from the master circadian pacemaker. *Neuroscience and Biobehavioral Reviews, 36*, 254-270. doi:10.1016/j.neubiorev.2011.06.003

Bolles, R. C. & De Lorge, J. (1962). The rat's adjustment to a-diurnal feeding cycles. *Journal of Comparative and Physiological Psychology, 55*, 760-762. doi:10.1037/h0046716

Boulos, Z. & Logothetis, D. E. (1990). Rats anticipate and discriminate between two daily feeding times. *Physiology & Behavior, 48*, 523-529. doi:10.1016/0031-9384(90)90294-E

Bradley, S. P. & Prendergast, B. J. (2014). Adaptation to short photoperiods augments circadian food anticipatory activity in Siberian hamsters. *Hormones & Behavior, 66*, 159-168. doi:10.1016/j.yhbeh.2013.10.008

Cain, S. W., Chalmers, J. A., & Ralph, M. R. (2012). Circadian modulation of passive avoidance is not eliminated in arrhythmic hamsters with suprachiasmatic nucleus lesions. *Behavioural Brain Research, 230*, 288-290. doi:10.1016/j.bbr.2012.02.022

Cain, S. W., Featherstone, R. E., & Ralph, M. R. (2011). Circadian modulation of amphetamine sensitization in rats does not require the suprachiasmatic nucleus. *Biological Rhythm Research, 42*, 267-273. doi:10.1080/09291016.2010.495839

Carneiro, B. S. & Araujo, J. F. (2009). The food-entrainable oscillator: A network of interconnected brain structures entrained by humoral signals? *Chronobiology International, 26*, 1273-1289. doi:10.3109/07420520903404480

Carr, J. A. R., Tan, A. O., & Wilkie, D. M. (1999). Further evidence that rats use ordinal timing in a daily time-place learning task. *Behavioural Processes, 48*, 35-48. doi:10.1016/S3076-6357(99)00074-1

Carr, J. A. R. & Wilkie, D. M. (1997). Rats use an ordinal timer in a daily time-place learning task. *Journal of Experimental Psychology: Animal Behavior Processes, 23*, 232-247. doi:10.1037/0097-7403.23.2.232

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., & Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes & Development, 14*, 2950-2961. doi:10.1101/gad.183500

Davidson, A. J., Poole, A. S., Yamazaki, S., & Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system? *Genes, Brain & Behavior, 2*, 32-39. doi:10.1034/j.1601-183X.2003.00005.x

Davidson, A. J., Stokkan, K., Yamazaki, S., & Menaker, M. (2002). Food-anticipatory activity and liver per1-luc activity in diabetic transgenic rats. *Physiology & Behavior, 76*, 21-26. doi:10.1016/S0031-9384(02)00680-7

Deibel, S. H. & Thorpe, C. M. (2013). The effects of response cost and species-typical behaviors on a daily time-place learning task. *Learning and Behavior, 41*, 42-53. doi:10.3758/s13420-012-0076-4

Dibner, C., Schibler, U., & Albrecht, U. (2010). The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annual Review of Physiology, 72*, 517-549. doi:10.1146/annurev-physiol-021909-135821

Eastman, C. I., Mistlberger, R. E., & Rechtschaffen, A. (1984). Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. *Physiology & Behavior, 32*, 357-368. doi:10.1016/0031-9384(84)90248-8

Eastman, C. & Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology & Behavior, 31*, 417-427. doi:10.1016/0031-9384(83)90061-6

Escobar, C., Cailotto, C., Angeles-Castellanos, M., Delgado, R. S., & Buijs, R. M. (2009). Peripheral oscillators: The driving force for food-anticipatory activity. *European Journal of Neuroscience, 30*, 1665-1675. doi:10.1111/j.1460-9568.2009.06972.x

Gallistel, C. R. (1990). *The organization of learning*. Cambridge, MA: MIT Press. doi:10.3758/BF03196076

Gould, J. L. (1987). Honey bees store learned flower-landing behaviour according to time of day. *Animal Behaviour, 35*, 1579-1581. doi:10.1016/S0003-3472(87)80038-6

Gritton, H. J., Kantorowski, A., Sarter, M., & Lee, T. M. (2012). Bidirectional interactions between circadian entrainment and cognitive performance. *Learning & Memory, 19*, 126-141. doi:10.1101/lm.023499.111

Gritton, H. J., Stasiak, A. M., Sarter, M., & Lee, T. M. (2013). Cognitive performance as a *zeitgeber*: Cognitive oscillators and cholinergic modulation of the SCN entrain circadian rhythms. *Public Library of Science ONE, 8*(2), 1-15. doi:10.1371/journal.pone.0056206

Harper, D. G., Volicer, L., Stopa, E. G., McKee, A. C., Nitta, M., & Satlin, A. (2005). Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. *The American Journal of Geriatric Psychiatry*, *13*, 359-368. doi:10.1097/00019442-200505000-00004

Harrison, J. M. & Breed, M. D. (1987). Temporal learning in the giant tropical ant, *Paraponera clavata*. *Physiological Entomology, 12,* 317-320. doi:10.1111/j.1365-3032.1987.tb00756.x

Jones, J. R., Tackenberg, M. C., & McMahon, D. G. (2015). Manipulating circadian clock neuron firing rate resets molecular circadian rhythms and behavior. *Nature Neuroscience, 18*, 373-375. doi:10.1038/nn.3937

Krebs, J. R. & Biebach, H. (1989). Time-Place learning by garden warblers (*Sylvia borin*): Route or map? *Ethology, 83*, 248-256. doi:10.1111/j.1439-0310.1989.tb00532.x

Liu, X. G., Zhang, B. J., Xu, X. H., Huang, Z. L., & Qu, W. M. (2012). Lesions of suprachiasmatic nucleus modify sleep structure but do not alter the total amount of daily sleep in rats. *Sleep and Biological Rhythms, 10*, 293-301. doi:10.1111/j.1479-8425.2012.00572.x

Lukoyanov, N. V., Pereira, P. A., Mesquita, R. M., & Andrade, J. P. (2002). Restricted feeding facilitates time-place learning in adult rats. *Behavioural Brain Research, 134*, 283-290. doi:10.1016/S0166-4328(02)00036-0

McDonald, R. J., Zelinski, E. L., Keeley, R. J., Sutherland, D., Fehr, L., & Hong, N. S. (2013). Multiple effects of circadian dysfunction induced by photoperiod shifts: Alterations in context memory and food metabolism in the same subjects. *Physiology & Behavior, 118,* 14-24. doi:10.1016/j.physbeh.2013.04.010

Mistlberger, R. E. (1994). Circadian food-anticipatory activity: Formal models and physiological mechanisms. *Neuroscience and Biobehavioral Reviews, 18,* 171-195. doi:10.1016/0149-7634(94)90023-X

Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M., & Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research, 739,* 12-18. doi:10.1016/S0006-8993(96)00466-0

Mistlberger, R. E., Kent, B. A., Chan, S., Patton, D. F., Weinberg, A., & Parfyonov, M. (2012). Circadian clocks for all meal-times: Anticipation of 2 daily meals in rats. *Public Library of Science ONE, 7*(2), 1-10. doi:10.1371/journal.pone.0031772

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation, 12,* 239-260. doi:10.1016/0023-9690(81)90020-5

Moura, C. A. & Luchiari, A. C. (2016). Time-place learning in the zebrafish (*Danio rerio*). *Behavioural Processes, 128,* 64-69. doi:10.1016/j.beproc.2016.04.007

Mulder, C. K., Papantoniou, C., Gerkema, M. P., & Van Der Zee, E. A. (2014). Neither the SCN nor the adrenals are required for circadian time-place learning in mice. *Chronobiology International, 31,* 1075-1092. doi:10.3109/07420528.2014.944975

Munn, R. G. K. & Bilkey, D. K. (2012). The firing rate of hippocampal CA1 place cells is modulated with a circadian period. *Hippocampus, 22,* 1325-1337. doi:10.1002/hipo.20969

Munn, R. G. K., Tyree, S. M., McNaughton, N., & Bilkey, D. K. (2015). The frequency of hippocampal theta rhythm is modulated on a circadian period and is entrained by food availability. *Frontiers in Behavioral Neuroscience, 9*, 1-13. doi:10.3389/fnbeh.2015.00061

Ohtsuka-Isoya, M., Hayashi, H., & Shinoda, H. (2001). Effect of suprachiasmatic nucleus lesion on circadian dentin increment in rats. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 280*, R1364-R1370. doi:10.1152/ajpregu.2001.280.5.R1364

Pendergast, J. S., Oda, G. A., Niswender, K. D., & Yamazaki, S. (2012). *Period* determination in the food-entrainable and methamphetamine-sensitive circadian oscillator(s). *Proceedings of the National Academy of Sciences of the United States of America, 109*, 14218-14223. doi:10.1073/pnas.1206213109

Pendergast, J. S. & Yamazaki, S. (2014). Effects of light, food, and methamphetamine on the circadian activity rhythm in mice. *Physiology & Behavior, 128*, 92-98. doi:10.1016/j.physbeh.2014.01.021

Pizzo, M. J. & Crystal, J. D. (2002). Representation of time in time-place learning. *Animal Learning & Behavior, 30*, 387-393. doi:10.3758/BF03195963

Pizzo, M. J. & Crystal, J. D. (2004). Evidence for an alternation strategy in time-place learning. *Behavioural Processes, 67*, 533-537. doi:10.1016/j.beproc.2004.06.004

Pizzo, M. J. & Crystal, J. D. (2006). The influence of temporal spacing on time-place discrimination. *Learning & Behavior, 34*, 131-143. doi:10.3758/BF03193189

Reebs, S. G. (1996). Time-place learning in golden shiners (Pisces: Cyprinidae). *Behavioural Processes, 36*, 253-262. doi:10.1016/0376-6357(96)88023-5

Reebs, S. G. & Lague, M. (2000). Daily food-anticipatory activity in golden shiners: A test of endogenous timing mechanisms. *Physiology & Behavior, 70*, 35-43. doi:10.1016/S0031-9384(00)00240-7

Stephan, F. K. (2002). The "other" circadian system: Food as a zeitgeber. *Journal of Biological Rhythms, 17*, 284-292. doi:10.1177/074873040201700402

Stokkan, K., Yamazaki, S., Tei, H., Sakaki, Y., & Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science, 291*, 490-493. doi:10.1126/science.291.5503.490

Thorpe, C. M., Deibel, S. H., Reddigan, J. I., & Fontaine, C. J. (2012). Strain differences in a high response-cost daily time-place learning task. *Behavioural Processes, 90*, 384-391. doi:10.1016/j.beproc.2012.04.004.

Thorpe, C. M. & Wilkie, D. M. (2007). Rats acquire a low-response-cost daily time-place task with differential amounts of food. *Learning & Behavior, 35*, 71-78. doi:10.3758/BF03196076

Van der Zee, E. A., Havekes, R., Barf, R. P., Hut, R. A., Nijholt, I. M., Jacobs, E. H., & Gerkema, M. P. (2008). Circadian time-place learning in mice depends on *Cry* genes. *Current Biology, 18*, 844-848. doi:10.1016/j.cub.2008.04.077

Wall, K., Lewis, L. M., Deibel, S. H., Hallett, D., Skinner, D. M., & Thorpe, C. M. (2019). Consistent meal times improve performance on a daily time-place learning task. *Behavioural Processes.* doi:10.1016/j.beproc.2019.01.002

Widman, D. R., Sermania, C. M., & Genismore, K. E. (2004). Evidence for time-place learning in the Morris water maze without food restriction but with increased response cost. *Behavioural Processes, 67,* 183-193. doi:10.1016/j.beproc.2004.04.001

Table 1

Oscillators available to each of the four groups: Group SCN / FEO, Group SCN / FEOx, Group SCNx / FEO, and Group SCNx / FEOx.

Oscillator	SCN / FEO	SCN / FEOx	SCNx / FEO	SCNx / FEOx
SCN	✓	✓	X	X
FEO	✓	X	✓	X

Table 2

Initial number of rats, number of rats excluded from analyses with reasons for exclusion, and final number of rats, by group.

Group	Initial number of rats	Reason for Exclusion			Total excluded	Final number of rats
		200 days or older at start of experiment	Started skip sessions before criterion reached	Problem with sectioning – could not verify lesion		
SCN / FEO	10	2	0	N/A	2	8
SCN / FEOx	9	2	0	N/A	2	7
SCNx / FEO	17	4	0	1	5	12
SCNx / FEOx	16	2	1	0	3	13

Table 3

Days to criterion, successfulness of the lesion, rhythmicity, and timing strategy employed by each rat, by group.

Rat	Days to Criterion (18/20)	Lesion	Rhythmic	Timing Strategy
		SCN/FEO		
1	24	-	No	Circadian
2	62	-	Yes	Circadian
3	85	-	Yes	-
4	53	-	Yes	Circadian
5	19	-	No	Circadian
6	88	-	No	-
7	25	-	Yes	Undetermined
8	51	-	Yes	Circadian
		SCN/FEOx		
9	57	-	Yes	Ordinal
10	86	-	Yes	Circadian
11	31	-	No	Ordinal
12	83	-	Yes	Circadian
13	37	-	Yes	Ordinal
14	66	-	No	Ordinal
15	30	-	No	Circadian

		SCNx/FEO		
16	37	Complete	Yes	Ordinal
17	89	Complete	No	-
18	87	Complete	No	-
19	89	Partial	No	Ordinal
20	54	Partial	Yes	Circadian
21	63	Partial	No	Ordinal
22	31	Partial	Yes	Circadian
23	27	Partial	Yes	Circadian
24	51	Partial	Yes	Circadian
25	27	Partial	No	Circadian
26	88	Partial	No	-
27	27	Miss	No	Circadian
		SCNx/FEOx		
28	41	Complete	No	Circadian
29	17	Complete	No	Circadian
30	84	Partial	No	Ordinal
31	60	Partial	Yes	Undetermined
32	22	Partial	No	Circadian
33	86	Partial	Yes	-
34	49	Partial	Yes	Ordinal
35	76	Partial	No	Ordinal
36	88	Miss	Yes	Ordinal

37	88	Miss	Yes	-
38	59	Miss	No	Ordinal
39	59	Miss	Yes	Ordinal
40	87	Miss	No	-

Note. Criterion was set at 18 correct trials out of 20.

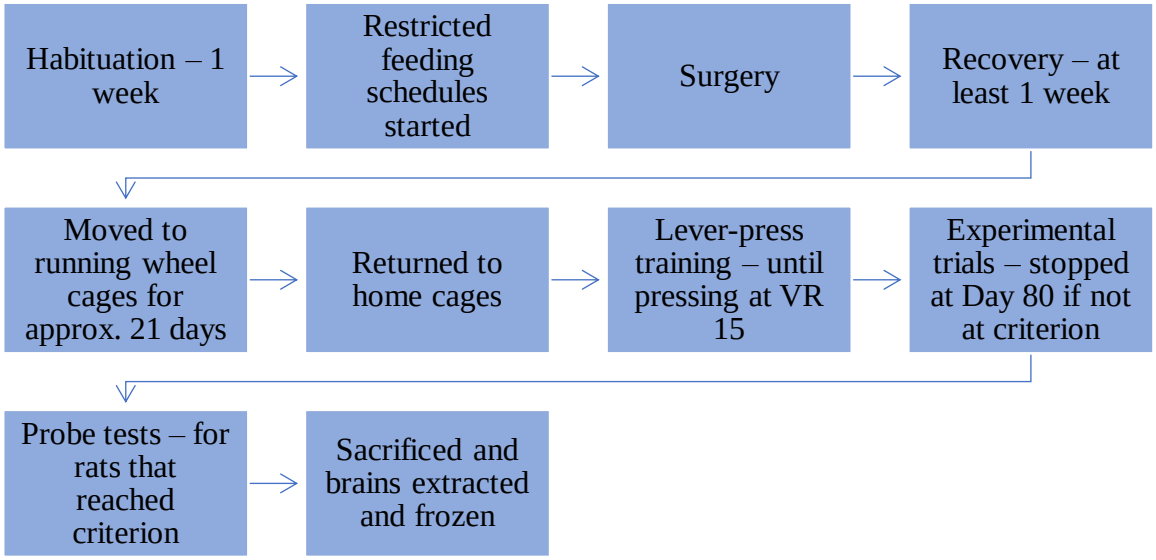


Figure 1. A timeline showing the procedure for rats. The length of some stages differed slightly for some rats, due to delays such as a rat requiring longer to recover from surgery than what was typical, or a rat taking longer to learn to lever press.

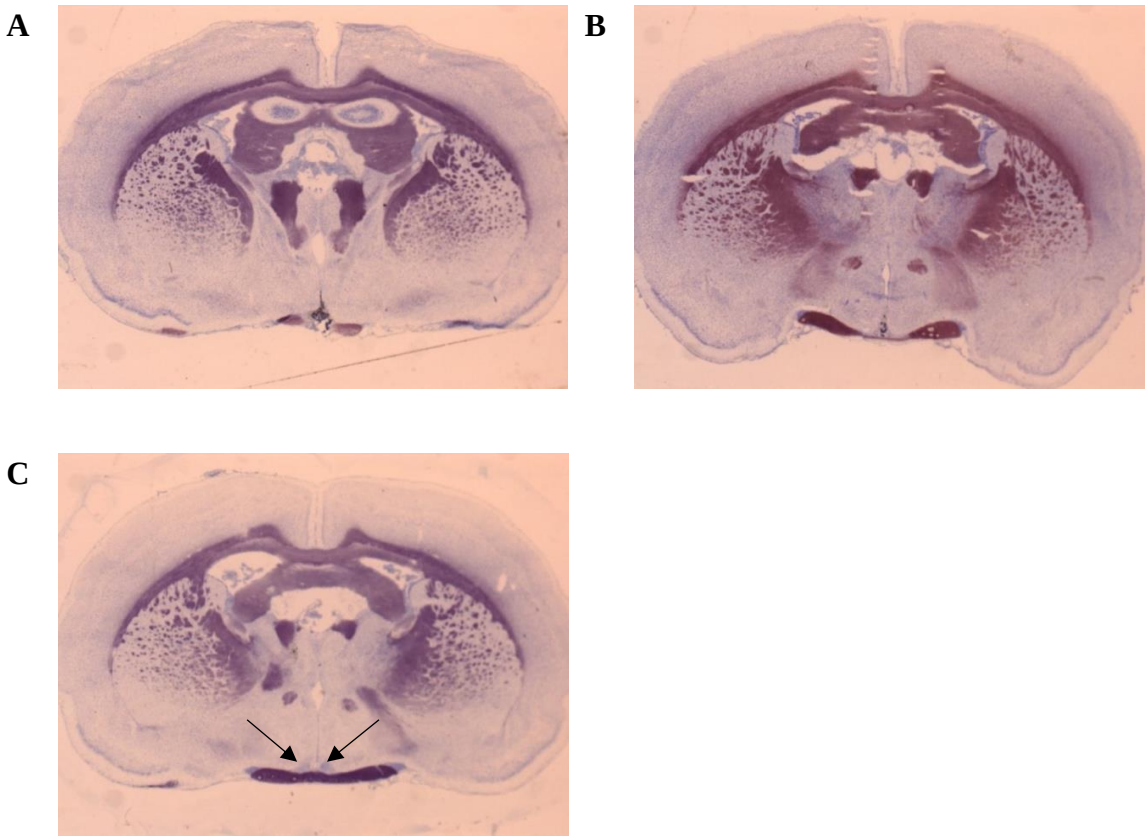


Figure 2. An image of a brain section from: A) a complete, B) a partial, and C) a sham lesion (arrows indicate the position of the SCN).

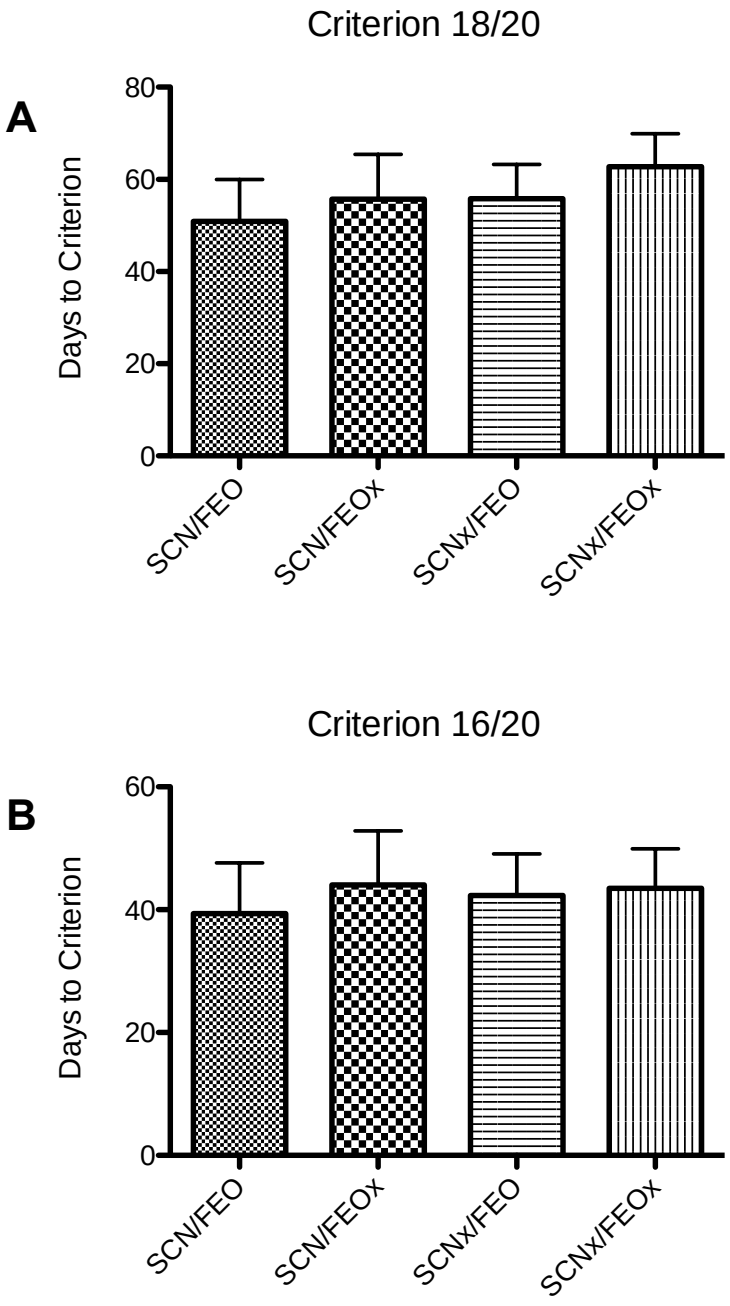
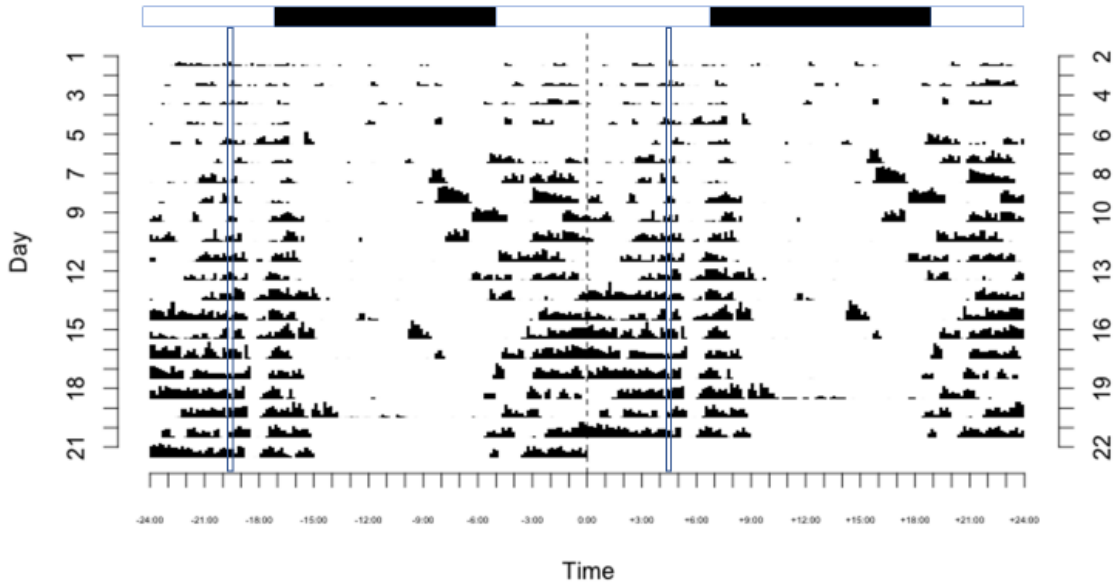
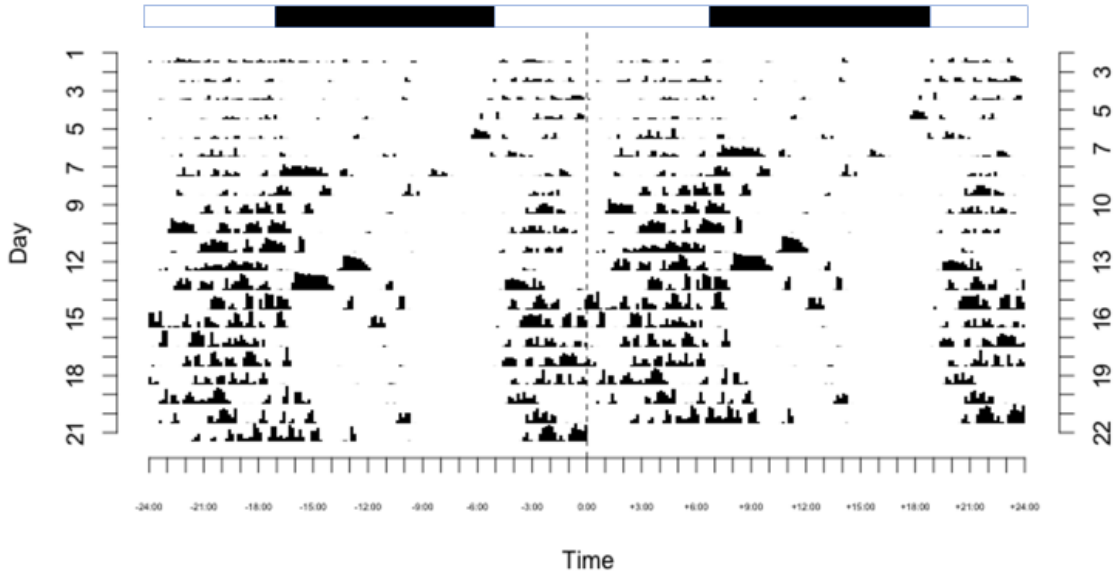


Figure 3. Mean days to criterion for each group with criterion set at: A) 18 correct trials out of 20, and B) 16 correct trials out of 20. Error bars represent the standard errors of the means.

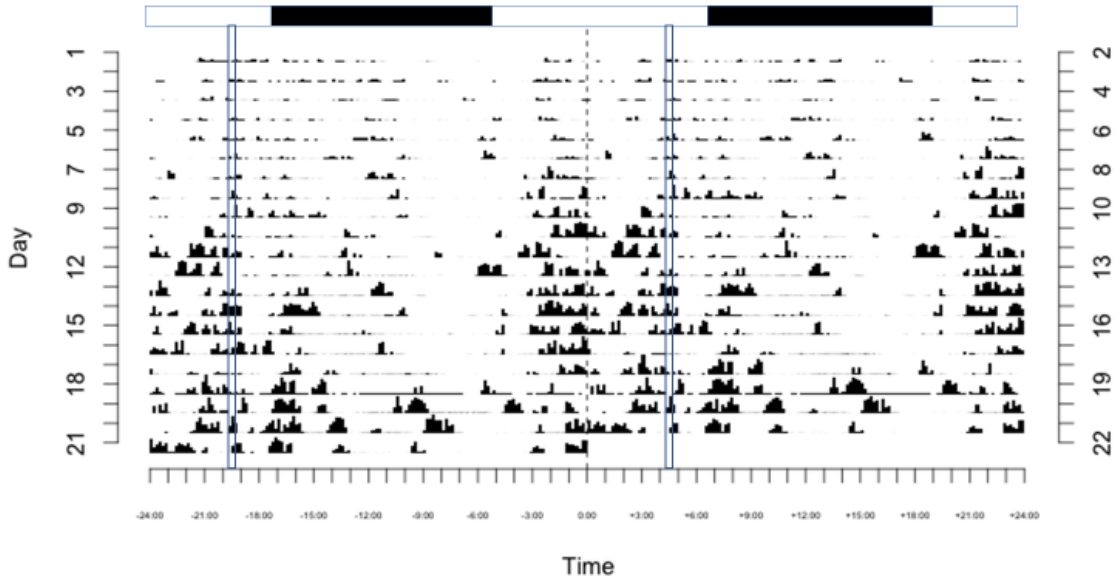
A



B



C


Day
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-21:00
-18:00
-15:00
-12:00
-9:00
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+3:00
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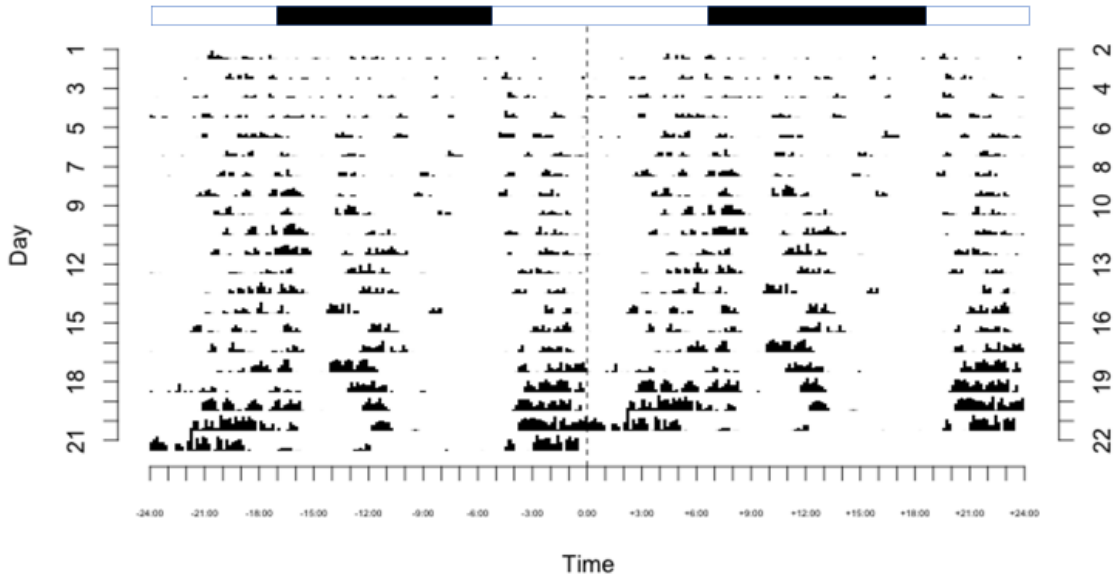
+9:00
+12:00
+15:00
+18:00
+21:00
+24:00
Time

D


Day
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+6:00
+9:00
+12:00
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+18:00
+21:00
+24:00
Time

Figure 4. A double-plotted actogram depicting running wheel activity of a rat in: A) Group SCN / FEO, B) Group SCN / FEOx, C) Group SCNx / FEO, and D) Group SCNx / FEOx. Horizontal bars across the top represent the light-dark cycle, with white bars representing the "lights on" periods and black bars representing the "lights off" periods. Vertical bars represent the daily feeding times (16:00) for rats with access to the FEO.