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Circadian time-place (or time-route) learning in rats with hippocampal lesions

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ABSTRACT

Circadian time-place learning (TPL) is the ability to remember both the place and biological time of day that a significant event occurred (e.g., food availability). This ability requires that a circadian clock provide phase information (a time tag) to cognitive systems involved in linking representations of an event with spatial reference memory. To date, it is unclear which neuronal substrates are critical in this process, but one candidate structure is the hippocampus (HPC). The HPC is essential for normal performance on tasks that require allocentric spatial memory and exhibits circadian rhythms of gene expression that are sensitive to meal timing. Using a novel TPL training procedure and enriched, multidimensional environment, we trained rats to locate a food reward that varied between two locations relative to time of day. After rats acquired the task, they received either HPC or SHAM lesions and were re-tested. Rats with HPC lesions were initially impaired on the task relative to SHAM rats, but re-attained high scores with continued testing. Probe tests revealed that the rats were not using an alternation strategy or relying on light-dark transitions to locate the food reward. We hypothesize that transient disruption and recovery reflect a switch from HPC-dependent allocentric navigation (learning places) to dorsal striatum-dependent egocentric spatial navigation (learning routes to a location). Whatever the navigation strategy, these results demonstrate that the HPC is not required for rats to find food in different locations using circadian phase as a discriminative cue.

1. Introduction

Circadian time-place learning (cTPL) is the ability to remember both the place and biological time of day that a significant event (e.g., food-availability) occurred. This ability is likely to be widely represented in the animal world, where optimal foraging requires attention to both time and place. cTPL has been demonstrated experimentally in a variety of species (e.g., insects, fish, birds, rats and mice), typically by providing a food reward at one time of day in one location, and at another time of day in a different location in a multi-chamber test environment (reviewed in Mulder, Gerkema, & Van der Zee, 2013). After training animals to some level of performance reliably better than chance, test trials are skipped to determine whether animals are learning an alternation rule (if fed in one of two locations in the morning, go to the other place in the evening) or are discriminating and remembering time of day (food is available in one place in the morning, and in the other place in the evening). To determine whether time of day is recognized using environmental cues (e.g., the light-dark cycle, LD) or internal time cues (e.g., the phase of a circadian clock), LD is replaced by constant light or dark for a day or more. These test conditions have confirmed that the phase of a circadian clock can be linked to memories of feeding events and places (Biebach, Falk, & Krebs, 1991; Boulos & Logothetis, 1990; Mistlberger, De Groot, Bossert, & Marchant, 1996; Mulder, Papantoniou, Gerkema, & Van Der Zee, 2014; Mulder, Reckman, Gerkema, & Van der Zee, 2015; Mulder et al., 2013; Saksida & Wilkie, 1994; Van der Zee et al., 2008).

The location of the circadian clock utilized for cTPL is unknown. The suprachiasmatic nucleus (SCN) is the site of a master circadian clock critical for entrainment of circadian rhythms to daily LD cycles, but is not required for entrainment to daily feeding cycles, or for accurate performance on TPL tasks (Boulos & Logothetis, 1990; Mistlberger et al., 1996; Mulder et al., 2014). A brain region hypothesized to play a critical role is the hippocampus (HPC) (Mulder, Gerkema, & Van der Zee, 2016). The HPC mediates

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allocentric spatial navigation and memory, and has been shown to express food-entrainable rhythms of circadian clock gene expression and other processes (Loh et al., 2015; Wakamatsu et al., 2001), indicating sensitivity to meal timing. The objective of the present study was to determine whether HPC lesions would disrupt performance on a cTPL task in rats. To test this, we developed a novel procedure and apparatus, in which rats are trained in a large multi-level environment to locate a food reward that varies between two locations according to time of day. To confirm that the rats were using time-of-day cues to correctly locate the food reward, rather than learning to alternate from one location to the other, we conducted probe tests that entailed omitting sessions and recording performance on the subsequent session. Rats that used a circadian strategy rather than simple alternation then received either HPC or SHAM lesions. To rule out the possibility that rats were discriminating intervals between LD transitions and test times to choose correctly, LD was replaced by constant dim red light (DDr) and the rats were tested for an additional two weeks.

2. Method

2.1. Subjects

The subjects were 20 experimentally naïve male Long-Evans rats (Charles River, St. Constant, QC), weighing 275–315 g at the start of the experiment. The rats were housed in polypropylene cages (48 × 25 × 20 cm) in a colony room maintained at 21 °C on a reverse 12:12 light-dark (LD) cycle, with lights off at 8:00 a.m., denoted Zeitgeber Time (ZT) 12, by convention. The rats had continuous access to water, and each received ~12 g of rat chow (Charles River Rodent Animal Diet, No. 5075) after each trial (i.e., mealtimes were ZT15.5 and ZT22). Prior to surgery, rats were pair-housed, and following surgery they were individually housed. All procedures were approved by the Concordia University Animal Care and Use Committee, and were in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Apparatus

Two large multi-level environments (152 × 145 × 86 cm) were used to test the rats (10 rats per apparatus; Fig. 1). Each apparatus was a modified, freestanding steel cage rack, enclosed on three sides by wire mesh, with a clear, removable Plexiglas front panel. Each apparatus had 4 levels. The lower 3 levels were divided into two equal halves by a plastic barrier wall, while the top level remained open. A loading cage (58 × 37 × 20 cm) was placed on top of the apparatuses. A rat entered the environment via a hole in the bottom of the loading cage that was placed over a passageway leading to the top level of the environment. The two food locations were on the lowest level (one on the left side of the barrier wall and the other on the right side). The 2 apparatuses were identical except for the conduits between the different levels of the environments. In Apparatus 1 (Fig. 1), rats traversed the environment vertically via wire mesh ladders located on both sides of the apparatus. In Apparatus 2, rats gained accessed to the different levels via short, vertical tubes (8 cm diameter) that were secured in the floor. There were 2 tubes on each level, 1 on each half of the level. In both environments, if a rat climbed down one side of the apparatus, it had to climb back up to the top level to traverse to the other side. Three different floor substrates (woodchip, wood pellets, and sand) were used to provide slight contextual differences between the two halves of the apparatus. Moreover, wire mesh boxes and wood logs were placed on certain levels to serve as local contextual cues. On the lowest level of Apparatus 1, there were additional divider walls that contained small swinging doors (10 cm diameter) that the rat had to pass through to obtain the food reward. The testing room contained dim red lights (4 lx) with a room temperature maintained at 21 °C. A video camera was positioned in front of each apparatus and test trials were video recorded for later analysis.

2.3. Surgery

Rats received excitotoxic lesions of the hippocampal formation (HPC group, $n = 5$), or sham surgery (SHAM group, $n = 4$). Isoflurane (0.8 L/min oxygen at 14.7 psi at 21 °C; Janssen, Toronto, ON) was used for anesthesia. The rats were secured in a stereotaxic frame and a midline scalp incision was made to expose the skull. The lesions were made by injecting N-methyl-D-aspartic acid (NMDA; Sigma-Aldrich, St. Louis, MO) at 10 sites, bilaterally (see Table 1 for coordinates based on Paxinos and Watson (1998)). A 26-gauge injection cannula connected to PE-20 tubing was attached to a 10 µl Hamilton syringe mounted on a micro-injection pump (KD Scientific). The NMDA (5.1 mM solution, dissolved in 0.1 M phosphate buffered saline) was infused at a flow rate of 0.15 µl/min until a total volume of 0.4 µl was reached at each site. The injection cannula remained in place for an additional 2.5 min before being retracted. Following the surgery, the incision was closed using wound clips and a topical antibiotic (Hibitane; Wyeth Animal Health, Guelph, ON) was applied to the incision area. Each rat received an injection of diazepam (10 mg/kg, ip; Hoffmann-La Roche, Mississauga, ON) as a prophylaxis against seizures. Sham-surgery rats underwent the same surgical procedure, without NMDA infusion. Injectors were lowered to 10 sites bilaterally and remained in place for 1 min. All rats received Penicillin G Procaine (0.2 ml, sc; Vétoquinol N.–A Inc., Lavaltrie, QC) and Ketoprofen (5 mg/kg, sc; Merial Canada, Baie d'Urfé, QC) post-surgery. The rats were given a 2-week recovery period prior to continuing behavioral testing. Food was provided ad libitum for the first 48 h after surgery, with the exception of one HPC rat that required wet food ad libitum for the first 72 h. For the remaining 12 days, the rats were fed 12 g of food twice/day at their usual meal times.

2.4. Behavioral procedures

A timeline of the experiment is provided in Fig. 2. Prior to the start of testing, the rats were handled for 10 min daily for 1.5 weeks. All testing occurred during the dark phase of the LD cycle. Testing occurred 7 days/week except during skip-trial probe testing, in which case the rats were tested 5–6 days/week. For all stages of testing, the rats were removed from their home cage and transported from the colony room to the test room in a large opaque plastic bin (23 × 61 × 40 cm). For the first 5 weeks of testing (denoted Test Week −4 to 0 in Fig. 2), the rats were tested in groups of 10. For the remaining test weeks, the rats were tested individually. Results are reported starting from the first week of individual testing (i.e., Test Week 1 and onwards in Fig. 2).

2.4.1. Pre-surgery TPL task acquisition

Rats received two daily trials, the first early in the dark period (ZT13.5–15.5, hereafter Trial 1) and the second late in the dark period (ZT20–22, hereafter Trial 2). On each trial, 7 Cheerios (650 mg, General Mills) were placed on the bottom level of the environment. On Trial 1 the Cheerios were located to the left of the barrier wall and on Trial 2 the Cheerios were located to the right of the barrier wall. At the start of a trial, a rat was placed in the loading cage and given a maximum of 15 min to make a choice. A choice was defined as the front 2 paws touching the floor of one of the food locations (for rats tested in Apparatus 1, this meant passing through the door). A correct choice was defined as going to the correct food