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Circadian rhythms accelerate wound healing in female Siberian hamsters

Erin J. Cable [a,*], Kenneth G. Onishi [a], Brian J. Prendergast [a,b]

[a] Department of Psychology, University of Chicago, Chicago, IL 60637, USA
[b] Committee on Neurobiology, University of Chicago, Chicago, IL 60637, USA

HIGHLIGHTS

- Circadian rhythms (CRs) in healing rates were documented in female Siberian hamsters.
- In nocturnal hamsters, skin wounds heal faster if they occur prior to the rest phase.
- Behaviorally circadian arrhythmic hamsters heal wounds slower, with no CRs in healing.

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ABSTRACT

Circadian rhythms (CRs) provide temporal regulation and coordination of numerous physiological traits, including immune function. CRs in multiple aspects of immune function are impaired in rodents that have been rendered circadian-arrhythmic through various methods. In Siberian hamsters, circadian arrhythmia can be induced by disruptive light treatments (DPS). Here we examined CRs in wound healing, and the effects of circadian disruption on wound healing in DPS-arrhythmic hamsters. Circadian entrained/rhythmic (RHYTH) and behaviorally-arrhythmic (ARR) female hamsters were administered a cutaneous wound either 3 h after light onset (ZT03) or 2 h after dark onset (ZT18); wound size was quantified daily using image analyses. Among RHYTH hamsters, ZT03 wounds healed faster than ZT18 wounds, whereas in ARR hamsters, circadian phase did not affect wound healing. In addition, wounds healed slower in ARR hamsters. The results document a clear CR in wound healing, and indicate that the mere presence of organismal circadian organization enhances this aspect of immune function. Faster wound healing in CR-competent hamsters may be mediated by CR-driven coordination of the temporal order of mechanisms (inflammation, leukocyte trafficking, tissue remodeling) underlying cutaneous wound healing.

1. Introduction

The immune system and the central nervous system (CNS) engage in multiple interactions (e.g., [6,27,41]). Although classically viewed as composed of substrates separate from those that reside in the CNS, the immune system and the brain consist of interacting units that regulate host defense in context [26].

Temporal context is important to behavior, metabolism, and immune function. Circadian timing markedly affects the immune system, a connection that has been revealed both under steady-state conditions and following disruptions to the circadian timing system. Several aspects of immunity are dependent on the output of the circadian system [11]. Leukocyte trafficking from the blood to peripheral tissues exhibits a strong circadian rhythm (CR), with concentrations of blood leukocytes high during the inactive period, and low during the active period [9]. The magnitude of infection-induced (e.g., treatment with bacterial lipopolysaccharide [LPS]) acute inflammatory responses and subsequent expression of sickness behaviors is dependent on circadian time or time of day [3,4,14,28,29].

Cytokine and sickness responses are altered following disruption of the circadian timing system (lesions: [16,54]; disruptive light treatments: [36]; simulated shift work: [15]). CRs in immunity are not limited to innate immune responses: T cell-dependent inflammation (a measure of the adaptive immune response) is greater when antigen exposure occurs during the rest as compared to the active phase [34].

Circadian regulation of the immune system becomes readily apparent following disruption of CRs. Chronic CR disruption is correlated with a higher rate of cancer [37,40,47], whereas stable CR entrainment is associated with increased quality of life and survival in cancer patients [18,31,48]. Repeated shifts in the light-dark cycle robustly increase LPS-induced mortality in mice [8]. In addition, dim light during the dark phase is sufficient to suppress adaptive and innate immune responses

* Corresponding author at: Institute for Mind and Biology, University of Chicago, 940 E. 57th St., Chicago, IL 60637, USA.
E-mail address: erinca@uchicago.edu (E.J. Cable).

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in Siberian hamsters [5]. Light treatments that induce a loss of behavioral CRs also eliminate CRs in leukocyte trafficking in hamsters [35]. We hypothesize that CRs in leukocyte trafficking may be of particular significance for innate immune responses to tissue damage and subsequent wound healing [30,42,53]. In mice, loss of the protein NONO, which is crucial to normal circadian clock function, results in disorganization of cellular proliferation and remodeling following a dermal incision [24].

Wound healing is an innate immune response that reflects the coordinated activity of the immune system (e.g., inflammation, leukocyte and cell migration, tissue remodeling; [17]). The phases of wound healing are overlapping, but each is defined by specific characteristics: an initial inflammatory response rapidly follows tissue damage and hemostasis. Neutrophils act via phagocytosis and secretion of antimicrobial molecules during the early inflammatory response to clear bacteria and foreign material from wounds, which is critical for subsequent healing [49]. Macrophage and lymphocyte infiltration in the inflammatory and proliferative phases continue the removal of foreign material, generate collagen, and recruit fibroblasts to the wound site for formation of an extracellular matrix and tissue repair [1,10,51]. Finally, epithelial remodeling completes the process of wound healing; this stage may occur over long timescales (i.e., months to years; [52]).

To better understand the effects of CRs on wound healing, we used a technique for inducing circadian disruption that differs from more common models (jet lag, clock gene mutations, bright constant light, brain lesions). In Siberian hamsters, a combination of light treatments (a nocturnal light pulse followed by a phase shift in the light-dark cycle; "DPS treatment") eliminates CRs in sleep and locomotor activity [43,45] in a subset of individuals. DPS treatment sufficient to induce circadian disruption also renders mediobasal hypothalamic clock gene expression (*per1*, *per2*, *bmal1*, and *cry1*) arrhythmic and suppressed in amplitude [13]. DPS has the advantage of allowing arrhythmic hamsters to remain undisturbed in a standard light-dark cycle, without the necessity of lesions or genetic manipulations.

Using this non-invasive method of inducing behavioral arrhythmia, these experiments examined the functional relevance of coherent behavioral CRs in the integrative immune process of cutaneous wound healing. This was accomplished using two convergent approaches: (1) examining healing rates of wounds delivered at different circadian phases in circadian competent hamsters, and (2) examining the effects of circadian disruption/arrhythmia on wound healing rates.

2. Methods

2.1. Animals

Female Siberian hamsters (*Phodopus sungorus*) were derived from a breeding colony maintained in a long-day, 15L:9D photoperiod (LD) at the University of Chicago. Hamsters were housed in polypropylene cages, with food (Harlan, Teklad) and filtered tap water provided *ad libitum*; cotton nesting material was available in the cages. Ambient temperature and relative humidity were held constant at 19 ± 2 °C and 53 ± 10%, respectively. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago. Hamsters were subjected to the circadian disruptive phase shift procedure ('DPS'; see Section 2.2) at 2–4 months of age.

2.2. Circadian rhythm disruption (DPS procedure)

The DPS manipulation that destabilizes the hamster circadian pacemaker employs phase-resetting light stimuli that render a proportion of hamsters behaviorally circadian arrhythmic ("ARR"; [43]). Hamsters for this experiment were drawn from a larger cohort of female hamsters that was subjected to the DPS procedure and then were assigned to different experiments. For the DPS procedure, hamsters were first housed for 4 weeks in a 16L:8D photoperiod. Then, on a single night, a 2 h light pulse was administered during the 5th through 7th h of the dark phase. On the next day, the 16L:8D photocycle was phase-delayed by 3 h, via extension of the light phase. Of the hamsters selected for the present study, 35 were subjected to the full DPS protocol, and 8 hamsters received a sham/control light treatment: they were subjected to the 3 h phase-delay, but were not given the 2 h light pulse on the prior night; this control manipulation does not lead to high rates of circadian disruption [43].

After DPS treatment, home-cage locomotor activity (LMA) was assessed using passive infrared motion detectors positioned outside the cage (22 cm above the cage floor). Motion detectors registered activity when 3 of 27 zones were crossed. Activity triggered closure of an electronic relay recorded by a computer running ClockLab software (Actimetrics, Evanston, IL). Cumulative activity counts were collected at 1 min intervals. Activity data for circadian chronotyping (see below) were collected in a single 10 day interval occurring 10–11 months after the DPS treatment, and 12–30 days before cutaneous wound healing was assessed (cf. [44]).

2.3. Circadian chronotyping following DPS

Criteria for assessing the presence/absence of CRs were similar to those in prior reports of DPS-induced CR disruption [43,44]. χ^2 periodogram and Lomb-Scargle periodogram (LSP) were used to detect presence/absence of CRs. LSP analyses were implemented to compliment χ^2 analyses, due to the increased sensitivity of the LSP in detecting CRs in non-sinusoidal data (e.g., 'square-wave' data, typical of circadian-entrained hamsters) and an increased propensity for χ^2 periodograms to indicate false peaks in noisy data (as is typical of DPS-induced ARR hamsters; [39,46]). χ^2 and LSP analyses were performed on a 10 day block of activity data (cf. [44]). CR amplitude was quantified in all hamsters, regardless of chronotype, as the Qp value at 24.0 h from the χ^2 periodogram.

Hamsters were designated as entrained (RHYTH) if they exhibited: (1) significant ($p < 0.001$) circadian (22–26 h) activity peaks in both the χ^2 periodogram and the LSP, (2) consistently clear daily activity onsets and offsets upon visual inspection of the actogram, and (3) activity predominantly restricted to the dark phase of the LD cycle.

Hamsters were designated as arrhythmic (ARR) if they exhibited: (1) the absence of clear and significant ($p < 0.001$) circadian peaks in χ^2 periodogram or the LSP, (2) an absence of consistent and clear daily activity onsets and offsets, and (3) LMA distributed throughout the light and dark phases of the LD cycle.

Three hamsters exhibited activity patterns that were not easily classifiable. One hamster exhibited a temporally-narrow (<1.8 min bandwidth) 'spike' in the χ^2 periodogram which did not resemble a normal circadian peak, but nevertheless exceeded the χ^2 significance level of $p < 0.0001$, indicative of power at a restricted frequency. This χ^2 'spike' exhibited no significant power in the surrounding frequency band (atypical of hamsters with normal CRs) and reached a peak amplitude of 8.9 Qp units above the significance level (cf., RHYTH hamsters exhibit peaks with a mean of 269.0 Qp units above the significance level). This animal also lacked clear circadian activity onsets and offsets, exhibited activity throughout the day and night, and exhibited almost no detectable power in the LSP (PN = 0.21) or the FFT (relative power = 0.001). For these reasons the hamster was classified as ARR. A second hamster exhibited modest but significant peaks in the χ^2 periodogram and the LSP, but lacked clear and consistent activity onsets and offsets, and exhibited locomotor activity throughout the light and dark phases on 4 of 10 assessment days, but temporal order in daily activity onsets and offsets on 6 of 10 days. A third hamster, although lacking significant peaks in the χ^2 periodogram and LSP, exhibited a free-running activity pattern ($\tau \sim 25$ h) with clear onsets and offsets on 7–8 of the assessment days. Due to their ambiguous and free-running chronotypes, respectively, these latter two hamsters were excluded from all analyses. 26 RHYTH and 14 ARR hamsters exhibited clear chronotypes and were used in these experiments.