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Circadian Disruption Alters the Effects of Lipopolysaccharide Treatment on Circadian and Ultradian Locomotor Activity and Body Temperature Rhythms of Female Siberian Hamsters

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Abstract

The effect of circadian rhythm (CR) disruption on immune function depends on the method by which CRs are disrupted. Behavioral and thermoregulatory responses induced by lipopolysaccharide (LPS) treatment were assessed in female Siberian hamsters in which circadian locomotor activity (LMA) rhythms were eliminated by exposure to a disruptive phase-shifting protocol (DPS) that sustains arrhythmicity even when hamsters are housed in a light-dark cycle. This noninvasive treatment avoids genome manipulations and neurological damage associated with other models of CR disruption. Circadian rhythmic (RHYTH) and arrhythmic (ARR) hamsters housed in a 16L:8D photocycle were injected with bacterial LPS near the onset of the light (zeitgeber time 1; ZT1) or dark (ZT16) phase. LPS injections at ZT16 and ZT1 elicited febrile responses in both RHYTH and ARR hamsters, but the effect was attenuated in the arrhythmic females. In ZT16, LPS inhibited LMA in the dark phase immediately after injection but not on subsequent nights in both chronotypes; in contrast, LPS at ZT1 elicited more enduring (~4 day) locomotor hypoactivity in ARR than in RHYTH hamsters. Power and period of dark-phase ultradian rhythms (URs) in LMA and T_b were markedly altered by LPS treatment, as was the power in the circadian waveform. Disrupted circadian rhythms in this model system attenuated responses to LPS in a trait- and ZT-specific manner; changes in UR period and power are novel components of the acute-phase response to infection that may affect energy conservation.

Keywords

immune function; circadian rhythms; ultradian rhythms; brain-immune interactions; sickness behavior

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CONFLICT OF INTEREST STATEMENT

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Immune function is modulated by the circadian pacemaker in the suprachiasmatic nucleus (SCN). Behavioral, thermoregulatory, and cytokine responses to innate immune challenges (acute-phase responses) reflect an interaction between peripheral cytokine production and brain responsiveness to cytokines (Dantzer et al., 2008). The acute-phase response may be particularly instructive for understanding interactions between the circadian and immune systems. Innate immune responses are robustly modulated by the circadian clock (Franklin et al., 2003; Marpegan et al., 2009): in many animal models, inflammatory responses to bacterial lipopolysaccharide (LPS) are greater during the interval approaching the onset of the active phase and reduced near the beginning of the rest phase (Curtis et al., 2014). Conversely, innate immune activation affects circadian timekeeping. LPS elicits SCN *c-fos* expression and shifts the circadian pacemaker (Marpegan et al., 2005). Convergent evidence suggests that LPS-induced phase shifts are mediated by proinflammatory cytokines (Marpegan et al., 2005; Paladino et al., 2010; Leone et al., 2012). Thus, time-of-day information derived from the circadian pacemaker modulates responsiveness of the immune system, and conversely, immune mediators modulate clock function.

Whereas circadian rhythms provide circa-24-h temporal order, ultradian rhythms (URs) impose essential temporal structure on subcircadian timescales (periods <8 h; Veldhuis, 2008; Yates and Yates, 2008). URs affect diverse aspects of physiology and behavior, including sleep (Mueller et al., 2012), hormones (Knobil, 1999; Choe et al., 2013; Lloyd et al., 2008), food intake (Warner et al., 2010), and body temperature (Heldmaier et al., 1989), traits that undergo striking changes during the acute response to infection (Hart, 1988). Despite the ubiquity of physiological and behavioral URs, it is unknown whether the temporal structure of URs, like circadian rhythm (CRs), is affected by immune activation.

Induced circadian arrhythmia has been widely used to assess circadian regulation of organismal physiology. Insights regarding circadian influences on immune responses have been gleaned from models of circadian arrhythmia induced by clock gene knockouts (Gibbs et al., 2012), constant illumination (LL; Deprés-Brummer et al., 1997), and ablation of the SCN (SCNx; Filipinski et al., 2003; Guerrero-Vargas et al., 2014), but each of these models has limitations. Clock genes are present in all tissues, and resulting immunophenotypes may reflect either arrhythmia or interference with cellular metabolic processes; LL can elevate immunosuppressive glucocorticoids (Welberg et al., 2006) and SCN lesions damage adjacent hypothalamic tissue, increase stress hormones (Buijs et al., 1993; Kalsbeek et al., 2012), and generate glial scars and neuroinflammation that persist for months after the insult (Logan et al., 1992; Silver and Miller, 2004). A general theme emerging from these and other studies is that the circadian system inhibits inflammatory responses and may provide a circadian temporal gating of inflammatory responses (Gibbs et al., 2012); other reports, however, indicate attenuated inflammatory responses in circadian-disrupted animals (Wachulec et al., 1997; Liu et al., 2006; Spengler et al., 2012).

Here we used a noninvasive model of circadian arrhythmia: the disruptive phase-shifted (DPS) hamster, which avoids the aforementioned complications, to examine the consequences of circadian disruption on innate inflammatory responses. DPS hamsters are well suited for translational analyses because, as in humans, dysrhythmia is manifested by genetically and neurologically intact individuals that remain exposed to circadian light-dark

cycles. In DPS hamsters, CRs in locomotor activity (LMA), T_b , sleep, hormone secretion, and SCN clock gene expression are eliminated by light treatments administered over the course of 2 days (Ruby et al., 2004; Fernandez et al., 2014).

The present experiment was conducted on female Siberian hamsters; females are understudied in neuroscience research (Beery and Zucker, 2011; Prendergast et al., 2014), an impediment to establishing brain-behavior relations applicable to both sexes.

MATERIALS AND METHODS

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 (Teklad; Harlan; Madison, WI) and filtered tap water provided ad libitum; cotton nesting material was available in the cages. Ambient temperature and relative humidity were held constant at $19 \pm 2^\circ\text{C}$ and $53\% \pm 10\%$, respectively. All procedures conformed with principles and guidelines in the *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Hamsters were subjected to the circadian DPS procedure at 2 to 8 months of age, and LPS treatments were administered between 8 and 15 months of age, after stable behavioral phenotypes had emerged. Because the DPS procedure induces circadian arrhythmia in a subset of hamsters (see below), middle-aged hamsters 8 to 15 months of age received the DPS treatment at one time point. In some reports, Siberian hamsters are regarded as “aged” at 17 to 24 months (Horton and Yellon, 2001; McKeon et al., 2011) and in others at 24 to 28 months (Reuss and Bürger, 1994; Reuss et al., 2000).

DPS Procedure

The DPS manipulation that destabilizes the hamster circadian pacemaker employs phase-resetting light stimuli that render a substantial proportion of hamsters permanently behaviorally circadian arrhythmic (“ARR”; Ruby et al., 2004) and disrupts SCN clock gene expression (Grone et al., 2011). Hamsters were housed for 4 weeks in a 16L:8D photoperiod, and then on a single night, a 2-h light pulse was administered during the fifth through seventh hours of the dark phase. The next day, the 16L:8D photocycle was phase-delayed by 3 h, by extending the light phase. Ninety-three hamsters were subjected to the DPS protocol, which typically renders >50% of hamsters permanently circadian arrhythmic (Ruby et al., 2004).

Locomotor Activity Monitoring

After DPS treatment, home-cage 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 were collected in consecutive

10-day blocks, over an interval spanning 2 to 3 months after the DPS treatment (cf. Ruby et al., 1998).

Circadian Phenotyping

Criteria for assessing the presence/absence of CRs were comparable to those in prior reports of DPS-induced CR disruption (Ruby et al., 2004; Ruby et al., 1998). The χ^2 periodogram analyses (ClockLab; Actimetrics) were performed on 10-day blocks of activity data (cf. Ruby et al., 1998). Hamsters were designated ARR absent clear and significant ($p < 0.001$) peaks in the periodogram in the circadian range, with LMA distributed throughout the light-dark cycle, and daily discrete activity onsets and offsets not evident on visual inspection of the periodogram; hamsters with significant circadian activity peaks in the χ^2 periodogram and clear daily activity onsets and offsets were considered entrained/ rhythmic (RHYTH) (Ruby et al., 1998). Sixteen RHYTH and 16 ARR hamsters were randomly assigned to receive LPS and saline treatments. LMA records of representative RHYTH and ARR hamsters are illustrated in Figure 1.

Surgical Procedures

Upon completion of circadian phenotyping, hamsters were anesthetized with isoflurane vapors (3% in medical O₂) and received precalibrated radio-telemetric transmitters intraperitoneally (i.p.) (Mini-Mitter ER-4000, E-mitters; Philips Respironics Murrysville, PA). Postoperatively, subcutaneous (s.c.) buprenorphine analgesic was administered at 12-h intervals for 48 h. E-mitters transmitted mean body temperature (T_b) and cumulative LMA at 6-min intervals to receiver boards placed under the cages. Temperature and LMA data were acquired using Vitalview software (Philips Respironics) and stored for offline analyses.

LPS Treatments

After a postsurgical recovery interval 1 month, physiological and behavioral sickness responses to LPS were evaluated as previously described (Prendergast et al., 2008). Estrous cycle (EC) stage is difficult to monitor in this species (Wynne-Edwards and Lisk, 1987); therefore, the day of the EC on which LPS was administered was not known and may have introduced some variability in the data; however, because all hamsters were freely cycling, any such error was randomly distributed across all subjects and all treatment conditions. Body mass and food intake values were recorded daily; body mass data are not reported here. After 3 d of baseline testing, hamsters were injected i.p. with either bacterial LPS (625 $\mu\text{g}/\text{kg}$; isolated from *Escherichia coli* strain 026:B6, Sigma, St. Louis, MO) or sterile 0.9% saline (0.1 mL). This dose of LPS is commonly used in studies of Siberian hamsters (Wen et al., 2007; Prendergast et al., 2008), which require a somewhat higher dose to elicit robust sickness responses (Bilbo et al., 2002, 2003; Prendergast et al., 2003). Injections in one cohort of hamsters were delivered at ZT16 (during 15 min immediately preceding the onset of darkness) and in a separate cohort at ZT1 (1 h after light onset). Food intake, body mass, T_b, and LMA were recorded for 6 to 7 d after injections. LPS and saline treatments were administered in a block-randomized design. Hamsters were randomly assigned to initial LPS/ saline treatment groups, and treatments were delivered in a counterbalanced design, with successive injections separated by 10 to 14 days. Due to equipment failure, T_b and activity data from a subset of RHYTH (LPS, $n = 4$; saline, $n = 4$) and ARR (LPS, $n = 4$;

Saline $n = 4$) hamsters injected with LPS at ZT1 were collected for 48 h after injection, but no T_b or activity data were recorded for the ensuing 4 days.

Analyses of Ultradian Responses to LPS Treatment

Time series associated with T_b and LMA were analyzed using methods typically applied to oscillatory neural signals (Kay and Beshel, 2010; Kay et al., 2009). Multitaper techniques, adapted to brief events in which only a small number of cycles may be present, were used to quantify URs and CRs. Ultradian and circadian frequency and power analyses were done in MATLAB using multitaper analysis tools (msspecgramc) from the Chronux toolbox (<http://www.chronux.org/>; Mitra and Bokil 2008). Ultradian spectral analysis was performed separately on 3 nonoverlapping 8-h windows to enable resolution of periods <4 h and to separate dark and light periods. Five tapers were applied to each 8-h data window, restricting the analysis band to $6.9e-5$ to $2.78e-4$ Hz (1- to 4-h periods). Power was estimated by summing the power within the analysis band for each 8-h interval. The dark and light phases were analyzed separately; however, complete data for light-phase activity and temperature URs are not reported here due to the absence of systematic differences between chronotypes, except as noted below. This summed power was then transformed to dB, as is standard procedure (Kay and Beshel, 2010), by $10 \cdot \log_{10}(\text{treatment power}/\text{baseline power})$, so that negative values represent a decrease and positive values an increase from the 8-d baseline means. The peak frequency was estimated using the pmtm multitaper function in MATLAB, and the maximum within the analysis band as described above was taken as the peak frequency.

Analyses of Circadian Responses to LPS Treatment

To assess CR power, a 2-day window (480 points at 6-min intervals) of T_b or LMA data, stepped by 1 day, was analyzed, resulting in 7 power analyses for an 8-d period. Preceding analysis, data were subjected to a 30-point moving average smoother, and then the mean of the 8 h of signal was subtracted. The multitaper analysis was performed using 5 tapers, a moving window of 480 points (48 h) stepped by 240 points (24 h); analysis was restricted to a band pass of 0.000005 to 0.00005 Hz (5.55- to 55.5-h periods). Preliminary analysis indicated that power from the smoothed signals fell within a restricted range, so power was summed within the period band 51.2 to 12.8 h. Power values were converted into dB using the mean baseline power for each hamster, as described above.

Statistical Analyses

Analyses of variance (ANOVAs) and post hoc pair-wise comparisons were performed with Statview 5.0 (SAS Institute, Cary, NC). Effects of injection treatment and circadian phenotype on T_b LMA and food intake were assessed using ANOVA followed by Fisher's protected least significant difference (PLSD) tests or 2-tailed t tests. To protect against type I error and α inflation in experiment 1, multiple pairwise comparisons of T_b were only performed after obtaining a significant omnibus F statistic, with α set to 0.01. In all other cases, differences were considered significant if $p < 0.05$.

RESULTS

Effects of Circadian Arrhythmia on Sickness Responses to LPS

Fever—LPS treatments at ZT16 elicited febrile responses, and circadian chronotype significantly modulated effects of LPS on the pattern of change in T_b (chronotype \times injection: $F_{49,1372} = 3.87$, $p = 0.001$; Fig. 2). Fevers persisted until the early hours of the next morning (Fig. 2A). During the ensuing dark phase, LPS-treated RHYTH hamsters exhibited hypothermia, whereas ARR hamsters exhibited no residual thermoregulatory response to LPS (Fig. 2C). In the next light phase, T_b was again elevated in RHYTH but not ARR hamsters treated with LPS.

LPS at ZT1 also elicited fever, and chronotype interacted with injection to affect the change in T_b ($F_{42,1176} = 1.51$, $p = 0.021$; Fig. 2B,D). In RHYTH hamsters, fever persisted for the first half of the light phase (Fig. 2B); during the ensuing dark phase, their nocturnal rise in T_b was reduced ($F_{1,14} = 6.92$, $p = 0.02$; Fig. 2B). Among ARR hamsters, LPS also caused fever for the first half of the initial posttreatment light phase, but thermoregulatory effects were not evident the following night (Fig. 2D).

Locomotor activity—In hamsters injected with LPS at ZT16, chronotype ($F_{119,3332} = 11.7$, $p = 0.001$) and injection ($F_{119,3332} = 1.72$, $p = 0.001$) affected the pattern of LMA over the next 5 days (Fig. 2); the chronotype \times injection interaction was marginally significant ($F_{119,3332} = 1.22$, $p = 0.054$). LPS inhibited LMA in RHYTH hamsters during the dark phase immediately after injection (0–8 h postinjection; $F_{1,14} = 46.3$, $p = 0.0001$, Fig. 2E), but on the ensuing 4 nights, LMA did not differ significantly between LPS- and SAL-treated hamsters. In ARR hamsters, LPS at ZT16 inhibited LMA on the night of injection ($F_{1,14} = 16.2$, $p = 0.0013$, Fig. 2G) but not thereafter.

LPS at ZT1 likewise inhibited LMA (chronotype: $F_{119,1428} = 3.52$, $p = 0.001$; injection: $F_{119,1428} = 1.46$, $p = 0.0013$; chronotype \times injection: $F_{119,1428} = 1.73$, $p = 0.001$; Fig. 2). Among RHYTH hamsters, LPS tended to decrease LMA during the first and second light phases postinjection ($F_{1,14} = 4.31$, $p = 0.057$, Fig. 2F) but not thereafter. LPS also inhibited activity during the first dark phase after treatment ($F_{1,14} = 18.3$, $p = 0.0008$), and nocturnal hypoactivity persisted for the next 3 nights ($F_{1,14} > 6.4$, $p = 0.04$, all comparisons; Fig. 2F).

In ARR hamsters, inhibitory effects of ZT1 LPS were not apparent during the light phase on any day after injection (Fig. 2H). In common with ZT16 injections, ZT1 LPS treatment decreased activity during the first dark phase postinjection ($F_{1,14} = 10.7$, $p = 0.0056$) but not during subsequent dark phases (Fig. 2H).

Anorexia—LPS reduced food intake ($F_{6,336} = 36.2$, $p = 0.0001$; Fig. 3), which depended on treatment ZT (injection \times ZT: $F_{6,336} = 2.81$, $p = 0.011$). Anorexic responses were reduced in hamsters injected with LPS at ZT1 relative to ZT16 ($F_{6,336} = 14.3$, $p = 0.0001$). LPS-induced anorexia at ZT16 was comparable in RHYTH and ARR hamsters (Fig. 3A, $F_{6,168} = 0.89$, $p = 0.51$). Food intake was lower in LPS-treated hamsters on posttreatment days 1 to 3 ($p = 0.013$, Fig. 3A), but not thereafter.

In contrast, LPS at ZT1 elicited different anorexic responses in RHYTH and ARR hamsters ($F_{6,168} = 3.44$, $p = 0.0031$). On day 1, LPS elicited anorexia in RHYTH hamsters (Fig. 3B, $p = 0.001$) but not ARR hamsters. Food intake in RHYTH ZT1-treated hamsters returned to normal values by the second posttreatment day (Fig. 3B).

Effects of LPS on Ultradian and Circadian Rhythms

UR power—ZT16 injections

Locomotor activity: Chronotype and injection interacted to affect power of dark-phase URs ($F_{6,168} = 3.13$, $p = 0.0062$; Fig. 4A). In RHYTH hamsters, LPS decreased UR power on the first 3 nights after treatment ($p = 0.009$, all comparisons; Fig. 4A) but did not significantly inhibit UR power in ARR hamsters (Fig. 4A).

Body temperature: LPS treatments affected the pattern of change in dark-phase T_b UR power ($F_{6,168} = 12.0$, $p = 0.0001$; Fig. 4B), but chronotype did not. On the second and third nights posttreatment, T_b UR power was reduced in LPS-treated RHYTH and ARR hamsters ($p < 0.05$, all comparisons; Fig. 4B).

UR power—ZT1 injections

Locomotor activity: LPS altered the pattern of change in dark-phase UR power over time ($F_{6,72} = 3.25$, $p = 0.007$; Fig. 4C), decreasing power on nights 1 to 4 after injection in RHYTH hamsters ($p < 0.05$, all comparisons) but only on night 1 ($p = 0.006$) in ARR hamsters (Fig. 4C).

Body temperature: LPS altered the pattern of change in dark-phase T_b UR power over time ($F_{6,72} = 7.46$, $p = 0.0001$; Fig. 4D), attenuating power on night 1 in both chronotypes ($p < 0.003$, both comparisons; Fig. 4D). UR power was reduced on night 2 in RHYTH ($p = 0.008$) but not ARR hamsters (Fig. 4D).

UR period—ZT16 injections

Locomotor activity: There was a significant main effect of circadian chronotype on dark-phase UR period (τ') ($F_{1,28} = 61.8$, $p = 0.0001$), with longer τ' s in saline-treated ARR than saline-treated RHYTH hamsters on most testing days (Fig. 5A). The effect of LPS on τ' was restricted to the dark phase immediately after treatment and was evident in RHYTH ($p = 0.0005$) but not ARR (Fig. 5A) hamsters.

Body temperature: Chronotype did not affect dark-phase T_b τ' (not illustrated). T_b τ' was not significantly affected by LPS treatment.

UR period—ZT1 injections

Locomotor activity: Chronotype significantly affected τ' of the dark-phase UR: periods were longer in ARR than RHYTH hamsters ($F_{1,12} = 26.9$, $p = 0.0002$; Fig. 5B). LPS, however, did not affect dark-phase τ' as a function of chronotype.

Body temperature: Dark-phase T_b τ' was not affected by chronotype or injection (not illustrated).

Circadian Rhythms

ZT16—CR power—LPS reduced circadian power in the LMA waveform ($F_{6,84} = 7.47$, $p = 0.0001$; Fig. 6A), with effects evident during the first and second 24-h intervals after injection ($p < 0.05$, both comparisons) but not thereafter. LPS also reduced T_b circadian power ($F_{6,84} = 10.4$, $p = 0.0001$; Fig. 6B) on the second through the fourth posttreatment days ($p = 0.002$, all comparisons).

ZT1—CR power

Locomotor activity: LPS decreased circadian power ($F_{6,36} = 3.68$, $p = 0.0059$; Fig. 6C) during the first 4 posttreatment days ($p < 0.05$, all comparisons) with a greater reduction in ZT1 than ZT16 hamsters on days 3 to 7 posttreatment ($p < 0.05$, all comparisons; Fig. 6A,C). In contrast, LPS at ZT1 did not affect power in the T_b circadian rhythm (Fig. 6D).

DISCUSSION

LPS-induced activation of the hamster innate immune system induced fever and sickness behaviors. Novel findings in the present report include evidence that power in locomotor activity (LMA) and T_b URs was enduringly altered, UR period was transiently lengthened, and CR power was chronically attenuated by LPS. Many of these responses were influenced by the timing of the immune challenge and/or the integrity of the circadian pacemaker. These data add to an emerging literature on effects of circadian organization and timing of immune challenges on multiple aspects of the acute-phase response to infection.

Despite unavoidable limitations in clock gene knockout, LL, and SCNx models of circadian arrhythmia, results from the DPS hamster model used here (which is genetically and neurologically intact) underscore a theme that has emerged from these other models, namely, that the circadian clock has important influences on the immune system. That these different approaches converge in establishing a strong circadian-immune connection suggests that effects observed after gene knockouts or SCN ablation may indeed reflect loss of timing information per se rather than pleiotropic effects or nonspecific lesion confounds.

Time of Injection Effects Were Dissimilar on LMA vs. T_b

The severity of the response to LPS varied over circadian time in a trait-specific manner. In RHYTH hamsters, LPS at ZT16 elicited more enduring febrile responses than at ZT1, whereas LMA responses were more persistent at ZT1 than at ZT16. These data add to work in rodents (Liu et al., 2006; Marpegan et al., 2009; Keller et al., 2009; Weil et al., 2009; Spengler et al., 2012) and humans (Petrovsky et al., 1998) documenting circadian variation in responses to LPS and other inflammatory challenges. The time of peak responsiveness to inflammogens depends on species, type of inflammatory challenge, and outcome measure.

A minority of responses to LPS in ARR hamsters varied as a function of injection ZT (e.g., anorexia). This may be attributable to masking effects of ZT treatment. There is precedent for circadian arrhythmic animals to exhibit trait-specific masking responses in a full L:D cycle: 42% of Syrian hamsters with large SCN lesions, behaviorally arrhythmic in running wheels in LL, synchronized locomotor activity, and ingestive behavior in the presence of a

light-dark cycle (Rusak 1977). Synchronization of wheel-running behavior by the light-dark cycle also is evident in multiple gene knockout mice (e.g., Bae et al., 2001; Izumo et al., 2014) that are circadian arrhythmic in constant darkness. Although LMA and T_b are arrhythmic in DPS hamsters exposed to a full light-dark cycle, a complete inventory of traits that exhibit masking responses to light (or darkness) in this model does not yet exist.

Circadian Arrhythmia Attenuated Behavioral and Thermoregulatory Responses to LPS

Compared to their respective saline-injected controls, T_b responses to LPS at ZT16 were attenuated in ARR compared to RHYTH hamsters, as was true of diminished LMA in response to LPS at ZT1. LPS has been reported to attenuate (Wachulec et al., 1997) or exacerbate (Guerrero-Vargas et al., 2014) T_b responses to LPS in SCNx rats. The dose of LPS and route of administration may account for these divergent results. Species and sex differences (Wachulec et al., 1997; Guerrero-Vargas et al., 2014) and the procedures employed to induce circadian arrhythmia also may affect responses to LPS. For example, the effects of circadian arrhythmia on memory processing differ categorically depending on whether arrhythmia is induced via SCNx or DPS (Fernandez et al., 2014). The direction and magnitude of effects of circadian arrhythmia on the inflammatory response to LPS may similarly depend on the methods employed to render animals arrhythmic.

Inflammatory responses in rodents with CR disruption induced by clock gene mutations likewise suggest that the method by which CRs are disrupted can affect the immunophenotype. Some circadian clock genes appear to enhance inflammatory responses to LPS: *Clock*-deficient mice exhibit reduced tumor necrosis factor α (TNF α)-mediated NF- κ B activation (Spengler et al., 2012), and *per2* mutant mice are resistant to LPS-induced sepsis (Liu et al., 2006). On the other hand, macrophages rendered arrhythmic via *bmal1*^{-/-} failed to exhibit CRs in LPS-induced interleukin (IL)-6 production, an effect also evident in macrophages from mice deficient in *rev-erba*; macrophages of *rev-erba*^{-/-} mice exhibit enhanced IL-6 responses to LPS (Gibbs et al., 2012). The macrophage clock likely inhibits cytokine production at certain circadian phases. Assessment of the proinflammatory cytokine responses to LPS in DPS-ARR hamsters would be instructive in ascertaining whether effects of chronotype reported here reflect ARR-induced decrements in peripheral production of inflammatory mediators.

Ultimately, disentangling the effects of loss of temporal order per se from the direct (noncircadian) effects of interventions that induce temporal disorder (Gibbs et al., 2012; Spengler et al., 2012; Sato et al., 2014) remains a challenge to understanding how the circadian timing system affects inflammatory responses and, more broadly, organismal-level immune function. Nevertheless, the present data, derived from neurologically and genetically “intact” hamsters, underscore the importance of the circadian system in the maintenance of normal immune responses.

LPS-Induced Inflammatory Responses Dampen Circadian Power

Multiple aspects of the circadian waveform changed after LPS treatment, which immediately decreased power of LMA activity CRs, regardless of injection ZT. Effects of LPS on power of CRs in T_b were only evident after ZT16 injections, however, and were delayed in

appearance. LPS causes modest but stable shifts in the phase of the circadian pacemaker in the SCN (Marpegan et al., 2005), effects mimicked by intracerebroventricular (i.c.v.) treatments with IL-1 β or TNF α , and blocked by inhibitors of TNF α signaling (Leone et al., 2012), suggesting that TNF α plays a central role in LPS-induced phase shifts. Decreases in overall LMA levels by LPS or live bacterial infections have been reported in several rodent models (reviewed in Harrington, 2012), as has inhibition by the viral mimetic Poly I:C (Katafuchi et al., 2005) and treatment with proinflammatory cytokines (e.g., IL-1 β ; Anisman et al., 2008). Initial global disruption of LMA, followed by a resumption of circadian activity patterns, is commonly reported (Harrington, 2012). Decreases in global LMA levels and decrements in the circadian waveform may reflect dampening of the underlying circadian pacemaker (Okada et al., 2008) and may constitute an underrecognized symptom of the acute-phase response to infection. Changes in CRs during acute illness may constitute an adaptive component of the acute-phase response to infection, conserving energy by decreasing the amount or amplitude of LMA and peak T_b.

Ultradian Rhythms Are Enduringly Altered by Inflammatory Responses

Effects of LPS on URs were evident in both RHYTH and ARR chronotypes, but there was a general pattern of diminished effects of LPS in ARR hamsters. In RHYTH females, the temporal structure (period) of URs was disrupted shortly after LPS treatment at ZT16. A ~1-h increase in the period of the LMA UR was evident during the first dark phase, immediately after injection. Normal UR periods were restored by the following night. Period-lengthening effects of LPS on LMA URs were not evident after ZT1 injections, and the periods of URs in T_b were unaffected by LPS. Thus, LPS has a potent, transitory period-lengthening effect on select URs and may be limited to inflammatory responses initiated around the time of activity onset.

In contrast, effects of LPS on UR power were evident at both ZTs; in many instances, decreases in UR power persisted for several days depending on trait and chronotype. At ZT16, LPS inhibited dark-phase LMA and T_b UR power of RHYTH hamsters for 2 to 3 nights. ZT1 LPS likewise inhibited UR power in both chronotypes, with more enduring effects in RHYTH hamsters.

To our knowledge, these are the first quantitative descriptions of changes in the UR waveform during the acute-phase response to simulated infection. Numerous reports describe effects of LPS on sleep (Krueger et al., 1986; Exton et al., 1995; Kapás et al., 1998; Schiffelholz and Lancel, 2001; Jakubcaková et al., 2011), a behavior with clear ultradian characteristics, but information on LPS-induced changes in the sleep ultradian waveform (period, power) is lacking.

URs are ubiquitous in vertebrate physiology, evident during both the active (food intake, LMA, T_b) and inactive (sleep, rest) phases. An important functional role for URs may lie in higher-frequency temporal partitioning of activity during each phase of the circadian cycle, conserving energy by consolidating behavior into bouts of relative activity and inactivity (Aschoff and Gerkema, 1985). Disorganization of ultradian temporal order normally present in well individuals may reflect novel, energetically costly aspects of the acute-phase response to infection.

Quantification of CNS cytokine or monoaminergic responses to LPS, although beyond the scope of the present study, could provide mechanistic insights into the chronobiological consequences of innate immune activation (cf. Blum et al., 2014).

Summary

In a novel model of circadian arrhythmia, with relevance to human circadian disruption, we investigated the role of circadian phase and integrity on multiple components of the behavioral response to infection. We confirmed and extended existing reports of circadian phase-specific effects of inflammation on sickness responses. Circadian arrhythmia attenuated several sickness responses, suggesting that temporal disorganization of the internal milieu in the DPS model may interfere with the generation of robust behavioral and immune responses to infection. The DPS treatment does not involve surgical or genomic manipulations; nevertheless, DPS-ARR hamsters exhibited altered inflammatory responses to LPS. These data offer novel, convergent evidence toward the importance of the circadian system in maintaining normal immune responses and suggest that immunological changes observed in knockout and lesion models may indeed be consequences of the elimination of circadian time information. Novel inhibitory effects of LPS on the generation of CRs and URs in multiple traits also were documented. Inflammation-induced changes in URs may reflect potentially costly components of the acute-phase response to infection.

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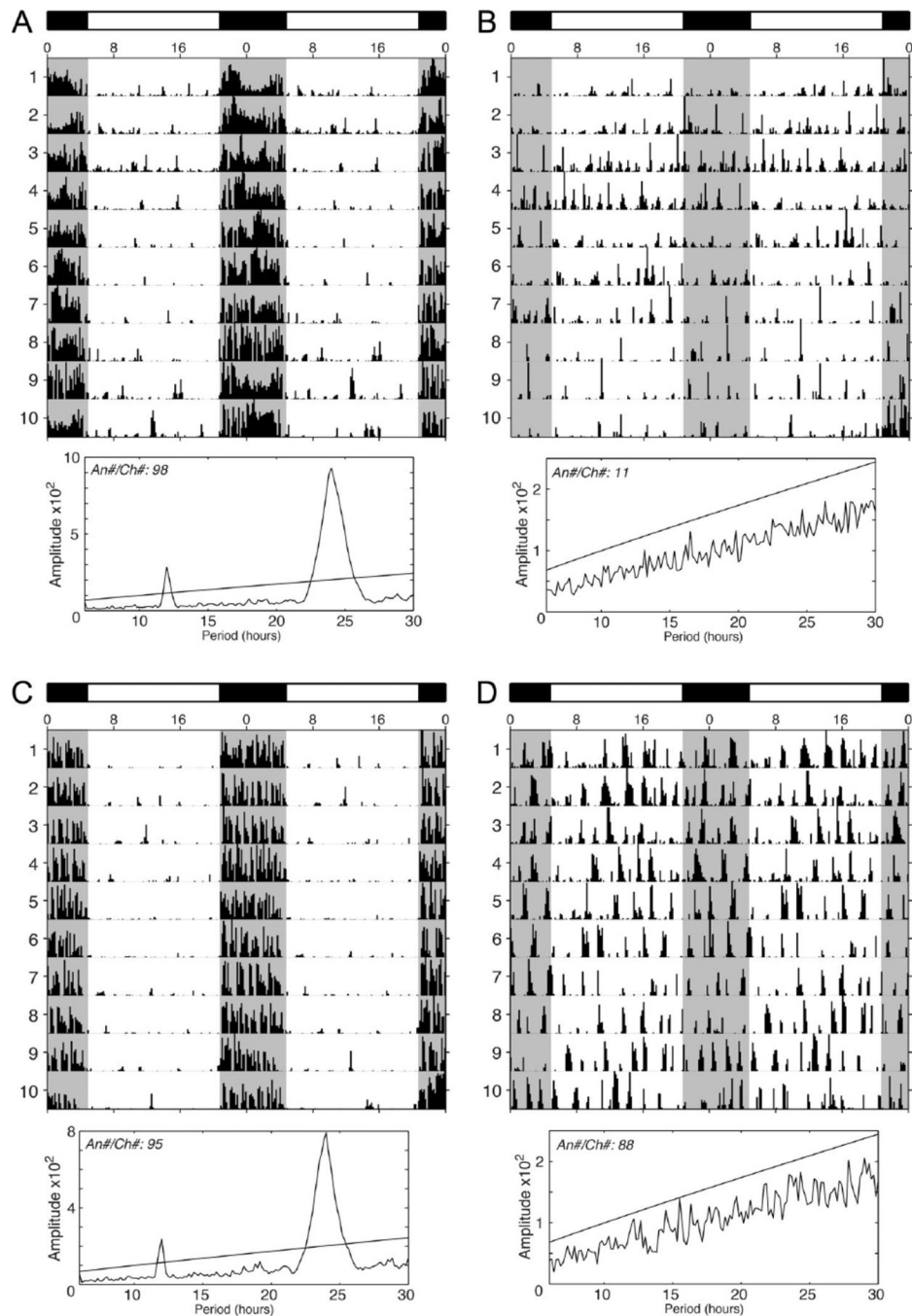


Figure 1. Disruptive phase shifts render hamsters behaviorally arrhythmic. Representative double-plotted activity records of circadian entrained hamsters (RHYTH; A and C) and of hamsters rendered circadian arrhythmic (ARR; B and D) by disruptive phase shift (DPS) light treatments (see “DPS Procedure” for details). Records depict 10 consecutive days of home-cage locomotor activity collected >1 month after the DPS procedure was applied; corresponding χ^2 periodogram analyses are depicted below each activity record. Values above the ascending line in the periodogram analysis indicate a significant ($p < 0.001$)

period. Time is indicated on the horizontal axis at the top of each actogram, along with light (white) and dark (black) phases of the 16L:8D photocycle. Shaded area indicates the dark phase.

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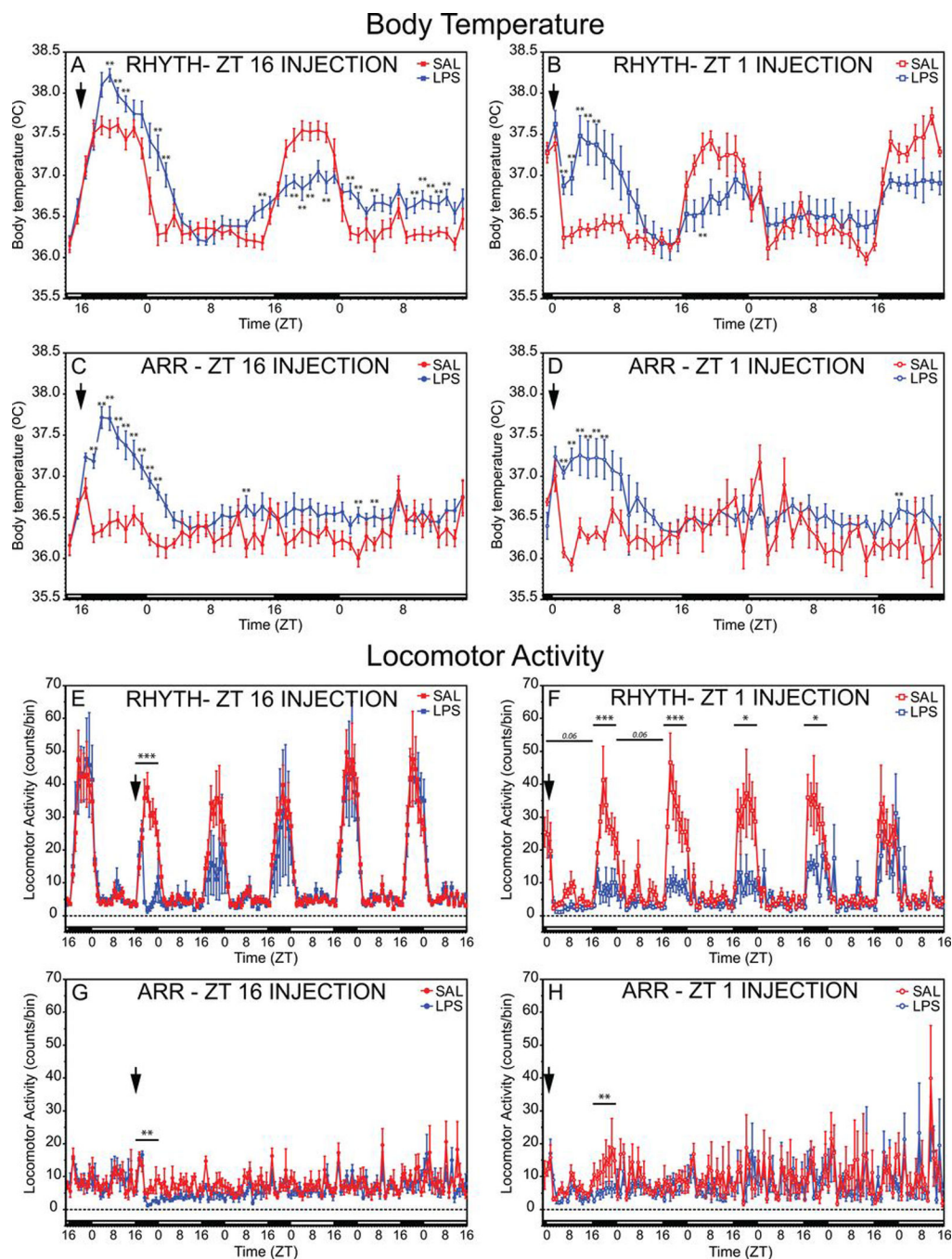


Figure 2. Lipopolysaccharide (LPS) elicits fever and inhibits locomotor activity. Mean \pm SEM body temperature (A–D) and spontaneous home-cage locomotor activity (LMA; E–H) of circadian entrained (RHYTH) and circadian arrhythmic (ARR) female Siberian hamsters injected intraperitoneally (i.p.) with bacterial LPS (625 μ g/kg) or sterile saline (SAL) at either ZT16 or ZT1. Chronotype and injection time are indicated within each panel. Each data point indicates the mean of 10 T_b values (6-min sampling interval) obtained during a given hour. The timing of injection treatments is indicated by a downward vertical arrow

near the ordinate axis, and the light/dark cycle is indicated with black (dark phase) and white (light phase) horizontal bars along the abscissa. A–D: $**p < 0.01$ vs. SAL value, within each panel. E–H: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. SAL value.

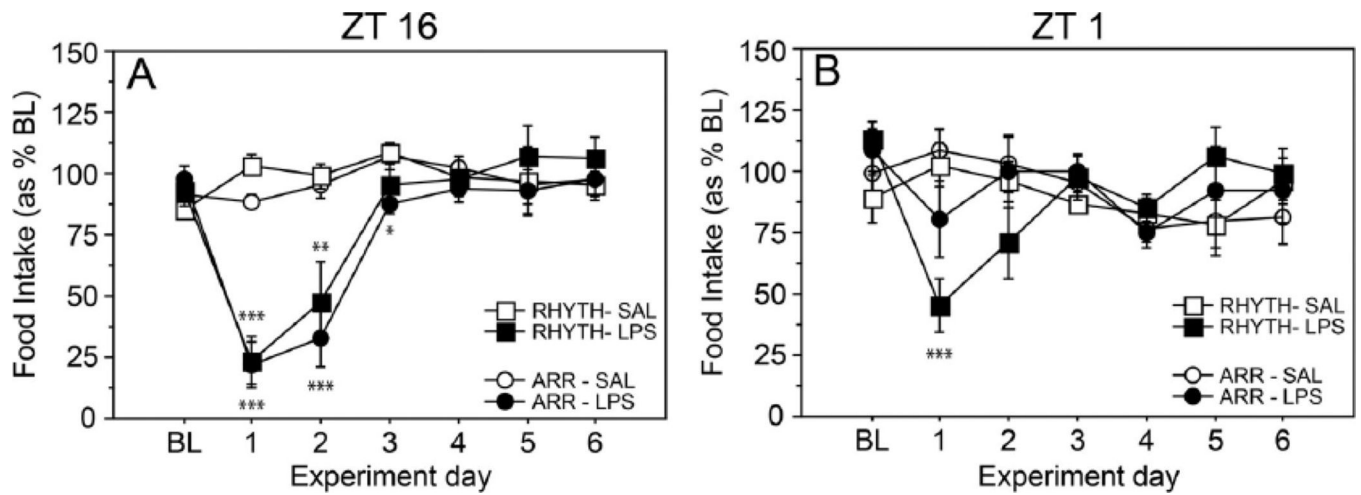


Figure 3.

Lipopolysaccharide (LPS)-induced anorexia. Mean \pm SEM change in food intake (A and B) of circadian entrained (RHYTH; squares) and circadian arrhythmic (ARR; circles) female Siberian hamsters injected intraperitoneally (i.p.) with bacterial LPS (625 μ g/ kg) or sterile saline (SAL) at either ZT16 (A) or ZT1 (B). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. SAL value, within chronotype. # p < 0.05 vs. RHYTH-LPS value.

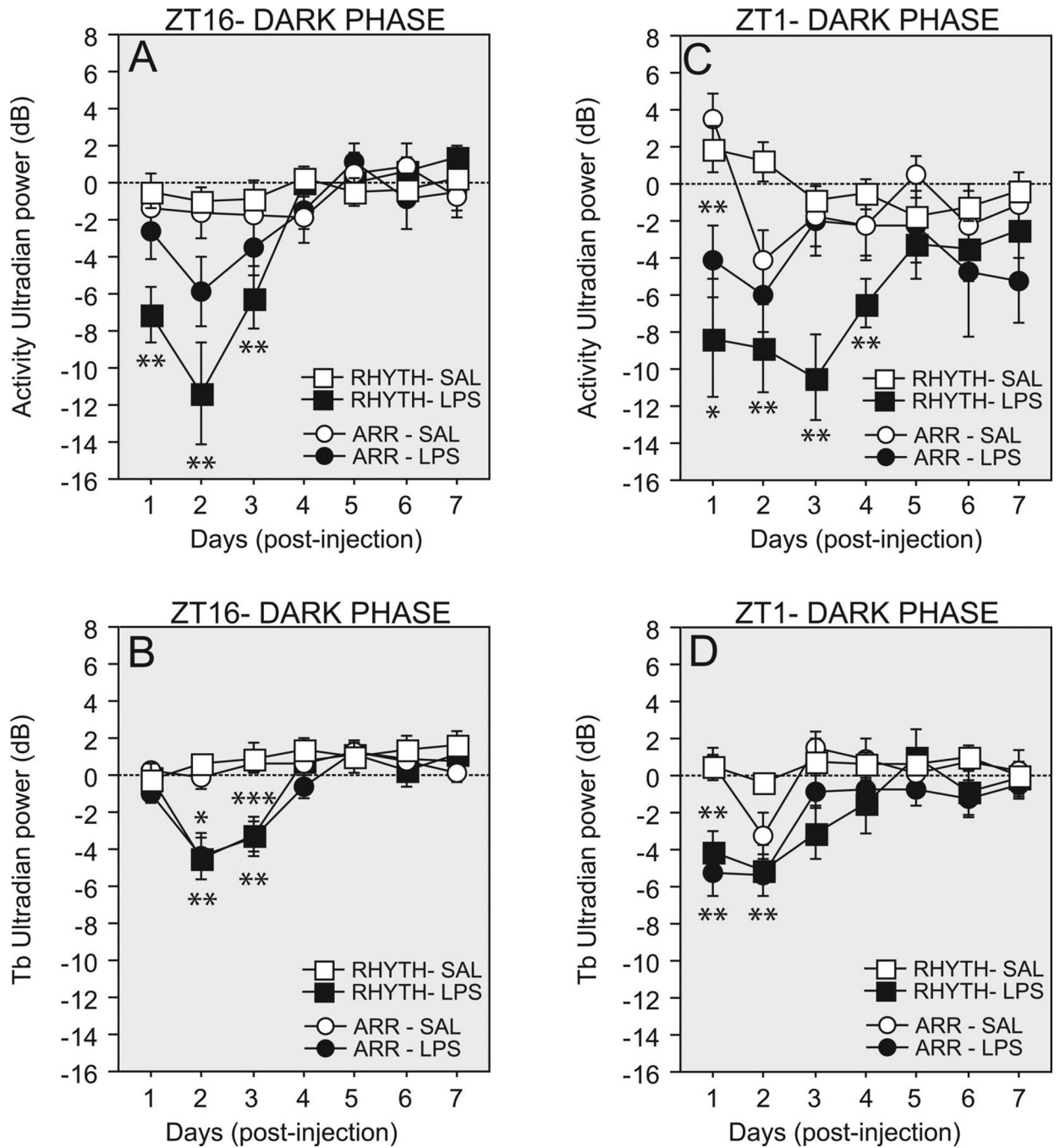


Figure 4. Lipopolysaccharide (LPS) decreases power of dark-phase ultradian rhythms. Mean \pm SEM power of the ultradian rhythm (UR) in locomotor activity (A and C) and body temperature (B and D) of circadian entrained (RHYTH; squares) and circadian arrhythmic (ARR; circles) female Siberian hamsters injected intraperitoneally (i.p.) with bacterial LPS (625 μ g/kg) or sterile saline (SAL) at ZT16 (A and B) or ZT1 (C and D). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. SAL value, within chronotype.

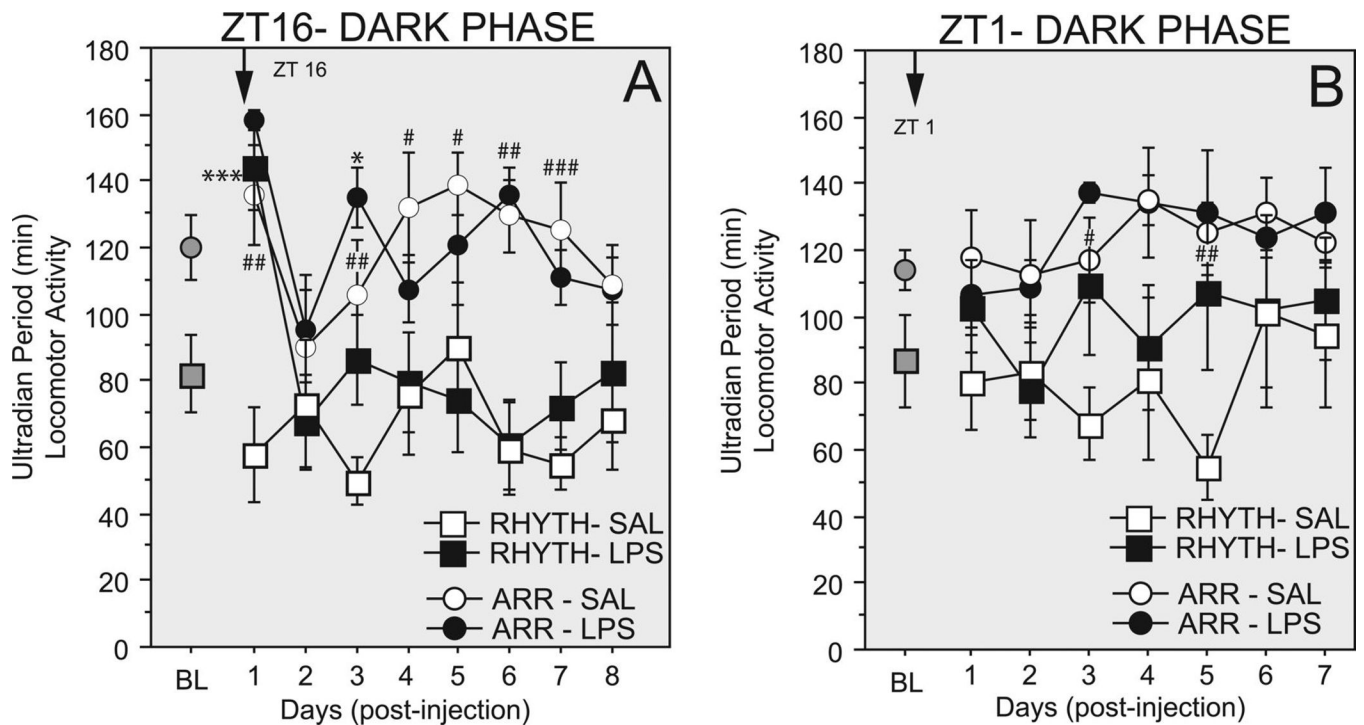
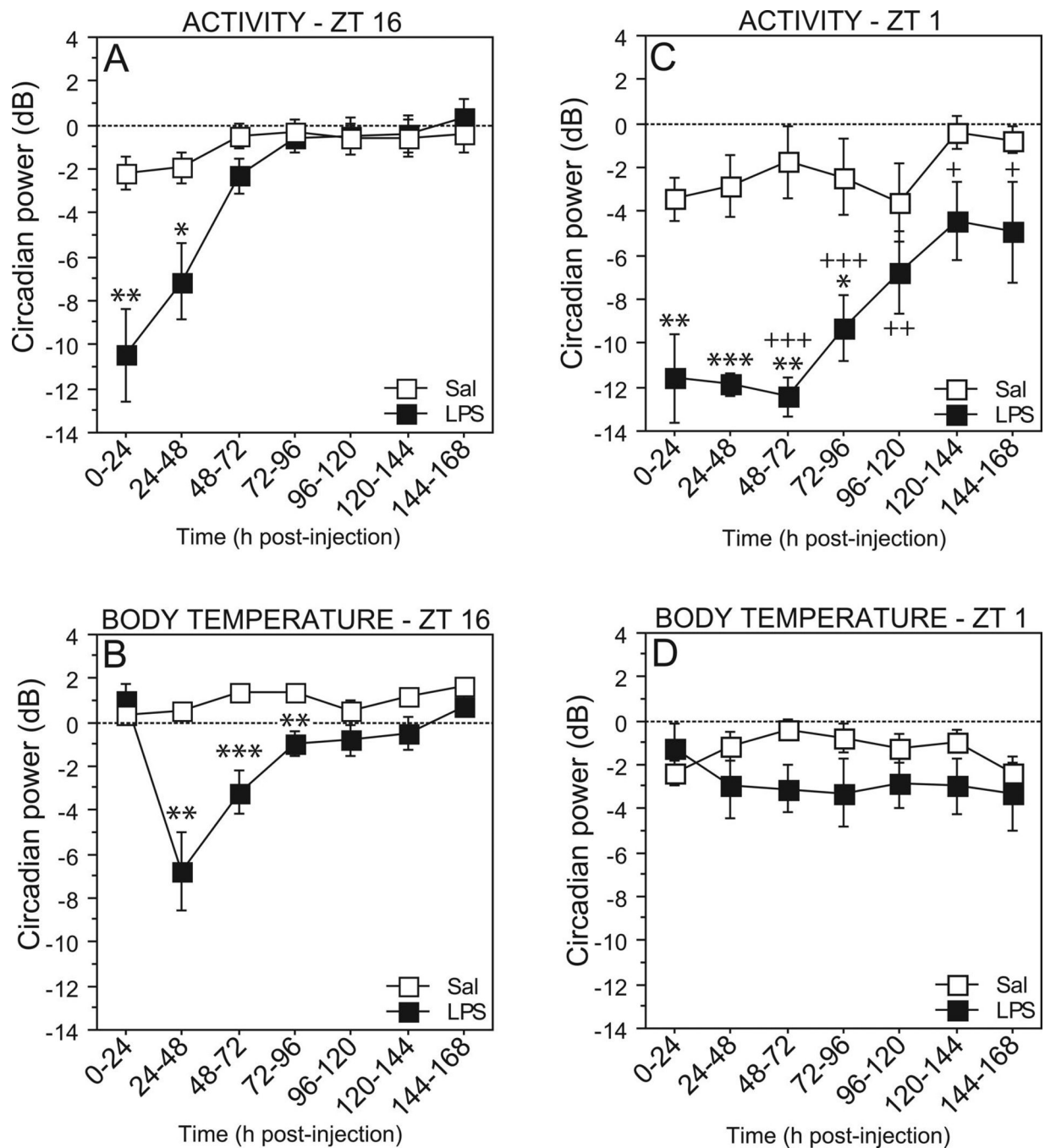


Figure 5.

Lipopolysaccharide (LPS) treatments decrease period of ultradian rhythms. Mean \pm SEM period of the ultradian rhythm (UR) in locomotor activity of circadian entrained (RHYTH; squares) and circadian arrhythmic (ARR; circles) female Siberian hamsters injected intraperitoneally (i.p.) with bacterial LPS (625 μ g/kg) or sterile saline (SAL) at ZT16 (A) or ZT1 (B). The timing of injection treatments is indicated by a downward vertical arrow near the ordinate axis. Gray symbols preceding injection treatments depict mean baseline values, averaged across the week prior to the initiation of injection treatments. * $p < 0.05$, *** $p < 0.001$ vs. SAL value, within chronotype; # $p < 0.05$, ### $p < 0.01$ RHYTH-SAL vs. ARR-SAL values.

**Figure 6.**

Lipopolysaccharide (LPS) inhibits power of behavioral and thermoregulatory circadian rhythms. Mean \pm SEM power of the circadian rhythm in locomotor activity (A and C) and body temperature (B and D) of circadian entrained (RHYTH) female Siberian hamsters injected intraperitoneally (i.p.) with bacterial LPS (625 μ g/kg) or sterile saline (SAL) at either ZT16 (A and B) or ZT1 (C and D). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. SAL

value. $^+p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ vs. LPS-ZT16 value for corresponding measure and time point.

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