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Title: Impact of Light at Night on Cardiac Arrest Outcome

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Introduction

The majority of permanent CNS damage resulting from cerebral ischemia is mediated by endogenous secondary processes (*1*, *2*). Although, neuronal cell death evolves over several days (*3*, *4*), the trajectory of recovery is likely determined early with acute inflammatory events participating in the secondary injury process (*5*, *6*). The delay in damage following cerebral ischemia provides a potential therapeutic window for reducing injury. This suggests immediate post-recovery environment may play a crucial role in establishing levels of neuroinflammation and neuronal damage that occur post-ischemia. Preventing CNS damage following cerebral ischemia influences long-term survival as well as cognitive and psychological outcome.

One environmental factor that is particularly variable in the hospital setting is nighttime light exposure. Hospital intensive care units have inconsistent levels of lighting during both day and night (*7*). These disruptive lighting conditions may influence patient recovery because light is the most potent entraining signal for the biological circadian clock (suprachiasmatic nuclei; SCN) in mammals; extrinsic light information travels directly from the retina to the SCN via the retinohypothalamic tract. Light input to the SCN coordinates internal signals, synchronizing daily physiological rhythms to the external light-dark cycle (*8*).

Circadian rhythms are important for homeostatic functions including those associated with the immune systems (*9*). There are circadian components to many immunological processes

including antigen presentation, toll-like receptor function, cytokine gene expression and lymphocyte proliferation (*10, 11*). Furthermore, many immune cells such as natural killer cells, macrophages, dendritic cells, and B cells possess molecular clock mechanisms necessary for self-sustaining oscillations (*12-14*). In healthy individuals, circadian misalignment can be rapidly induced with aberrant lighting schedules, resulting in adverse metabolic and cardiovascular consequences (*15*) . Individuals chronically exposed to LAN are at increased risk for heart disease (*16*), cancer (*17, 18*), disrupted rhythmicity of neuroendocrine function (such as corticotrophin releasing hormone, glucocorticoids, and prolactin) (*19, 20*), and mood disorders (*21*); a common risk factor in each of these pathologies is elevated inflammation. Moreover, shift workers have altered immune parameters including elevated C-reactive protein and increased leukocyte count (*22*). Importantly, circadian disruption dysregulates inflammatory responses independently of sleep loss or stress (*23*).

Cardiac arrest has both seasonal (i.e. day length) and circadian (i.e. time of day) patterns of incidence and recovery (*24-26*), suggesting a role for light in altering the physiological response to cerebral ischemia. Light exposure may be particularly salient to CA-induced neuroinflammation. Although several mechanisms contribute to damage following ischemic injury, including energetic failure, excitotoxicity, and oxidative damage (reviewed in (*5, 6*), manipulation of inflammatory responses are considered a prime target for improving recovery. Following CA an inflammatory response is triggered by activation of microglia and astrocyes with a corresponding upregulation of pro-inflammatory cytokines (*27*). IL-1β and (TNFα) are pro-inflammatory cytokines which are upregulated within hours following global ischemia (*1*) and exacerbate injury. For example, increased levels of hippocampal TNFα and IL-1β after CA are associated with elevated neuronal cell death one week later in a mouse model of CA (*25*).

Administration of mesenchymal stromal cells into the hippocampus following global ischemia decreases expression of many genes involved in inflammation and immune responses and reduces neuronal death (*28*). Likewise, inhibition of cyclooxygenase-2 (COX2), a mediator of inflammatory activity, decreases CA1 neuronal death resulting from cerebral ischemia (*29, 30*).

Dim light at night disrupts the circadian system and immune function implicating it as a critical factor for consideration in cardiac arrest outcome. We hypothesized that light at night would potentiate injury following cardiac arrest. Here we show the neurobiological effects of environmental lighting on CA outcome. Consistent with our hypotheses, light at night following CA enhanced acute cytokine responses, increased neuronal death, and resulted in higher short-term mortality. Moreover, the effects of light at night were ameliorated through inhibition of cytokines and manipulation of the light source.

Results

Hospital lighting levels

In order to assess typical lighting environments for patients recovering in hospital settings, HOBO light loggers (Onset, Bourne, MA) were placed in patient rooms at The Ohio State University Wexner Medical Center Hospital. Significant nighttime light intrusions were observed in all units monitored, including the intensive care, post-surgical, and cardiac intensive care units (Fig. 1a; representative patient lighting). Patients experienced light levels as high as 100 lux several times each night between the hours of 11PM and 6AM. After confirming nighttime light disruptions in patient rooms, we used a mouse model of CA to determine whether exposure to dim light at night influences recovery following global cerebral ischemia.

Dim light at night increases mortality and hippocampal damage following CA

Eight week old Swiss Webster mice were acclimated to a standard light dark cycle [14h light (150 lux): 10h dark (0 lux); LD] and then underwent a CA or SHAM procedure (Fig. S1). Following the procedure mice either remained in LD cycle or were transferred to a bright light-dim light cycle [14h light (150 lux): 10h dim light (5 lux); dLAN]. As anticipated, there was 100% survival in both SHAM groups. In contrast, CA significantly reduced survival compared to the SHAM procedure ($p < 0.05$; Fig 1b) and among the CA mice, mortality in the dLAN group was four-fold higher than in the LD group. These data suggest that modest changes in the recovery environment affect cardiac arrest survival.

The reduced survival rate of mice exposed to dLAN may reflect increased neuroinflammation and hippocampal cell death. One week following CA or SHAM procedures mice were anesthetized and perfused transcardially with ice cold saline followed by 4% paraformaldehyde. Brains were cryoprotected, frozen, sectioned, and processed for Fluoro-JadeC, a marker for degenerating neurons. The Fluoro-JadeC labeled tissue was used to evaluate cell death in the hippocampus, a brain region particularly vulnerable to ischemic damage (*31*). As expected, cell death was uniformly low among SHAM mice (Fig. 1c,d), and significantly elevated among mice subjected to CA (Fig. 1 e,f,g). Moreover, mice exposed to dLAN had significantly more cell death in the hippocampus one week after CA compared with mice exposed to dark nights (Fig. 1c-g). Hippocampal cell death is an excellent proxy for overall recovery after global ischemia as increased hippocampal damage is associated with elevated mortality and memory deficits, as well as impaired affective responses (*32-36*).

Dim light at night alters acute inflammatory status

The inflammatory response following global ischemia is an important factor in recovery. Thus, we investigated whether dLAN alters the expression of pro-inflammatory cytokines

following CA. Brain tissue was collected 24 h after CA or SHAM procedures, i.e. after only a single night of post-ischemic dLAN or LD. The brains were rapidly removed and placed in RNA later. The following day, hippocampi were dissected out and used for quantitative real time PCR (qRT-PCR) analyses of pro-inflammatory cytokines. As expected, TNFα, IL1β, and IL6 gene expression were elevated among CA compared to SHAM mice ($p < 0.05$; Fig 2a-c). Moreover, exposure to a single night of dLAN after CA was sufficient to upregulate expression of TNFα and IL1β, compared to mice exposed to a dark night ($p < 0.05$; Fig. 2a,b). The increase in pro-inflammatory cytokine expression may occur very early after placement back in lighting conditions. Indeed, a difference in post-CA TNFα expression was apparent after as little as 4 hr of total darkness versus dim light ($p < 0.05$; Fig. 2d). There were no differences in hippocampal cytokine gene expression between dLAN and LD mice that underwent the SHAM procedure ($p > 0.05$). In sum, acute upregulation of inflammatory markers among CA mice housed in dLAN may contribute to the increased hippocampal neuronal death and mortality observed in this group.

Microglia are the resident immune cells in CNS and perturbations of the microenvironment can induce microglial activation, resulting in altered morphology and secretion of pro-inflammatory mediators (*37, 38*). Therefore, we hypothesized that microglia increase cytokine expression 24 h after CA in dLAN mice, contributing to the overall elevation in inflammatory status in the dLAN-CA group. Microglia were extracted from whole brain tissue using a Percoll gradient 24 h after CA or SHAM procedures (a single night of dLAN or LD lighting conditions). mRNA was extracted immediately following microglial isolation and pro-inflammatory cytokine expression was evaluated. IL-1β and TNF-α mRNA expression was significantly higher in microglia isolated from CA mice as compared to SHAM mice ($p < 0.05$;

Fig. 2e,f). Furthermore, exposure to a single night of dLAN after CA elevated microglial IL-1β and IL-6 mRNA relative to LD ($p < 0.05$; Fig 2f,g). These results suggest that microglia may be partially responsible for the proinflammatory bias observed among mice that were housed in dLAN after CA.

We also examined whether altered circulating glucocorticoid concentrations could be contributing to the impaired recovery after CA because elevated corticosterone has previously been associated with increased CA-induced neuroinflammation and neuronal death (*39*). However, altered corticosteroid responses do not appear to underlie the differences in ischemic outcome between the CA-LD and CA-dLAN groups; there were no differences in corticosterone concentrations between CA or SHAM groups at 24h ($p > 0.05$; Fig. 2h). Moreover, investigation of corticosterone concentration at 6 hr intervals following CA did not reveal any significant differences between the CA-dLAN and CA-LD groups during the first 24 h of recovery ($p > 0.05$; Fig. S2).

Inhibition of selective pro-inflammatory cytokines ameliorates light induced damage

Although several mechanisms contribute to damage following ischemic injury, including energetic failure, excitotoxicity, and oxidative stress (reviewed in (*6*), manipulation of inflammatory responses are considered a prime target for prevention of damage. Following ischemic brain damage both selective (against specific cytokines; e.g., IL1 receptor antagonist (IL1-ra) administration) and non-selective (e.g. minocycline) inhibition of pro-inflammatory cytokines ameliorate damage improving recovery and behavioral outcomes (*39-42*). Because our results indicate that IL-1β, TNF-α, and IL-6 mRNA expression is greater among CA-dLAN mice compared to CA-LD mice, we hypothesized that selective inhibition of these pro-inflammatory cytokines would improve outcome following CA-dLAN. Three days prior to the CA procedure,

mice were implanted with a cannula directed at the lateral ventricle. Two hours following CA, mice were administered a single 2μL ICV injection of either the vehicle (artificial cerebrospinal fluid; aCSF), mouse IL6 neutralizing antibody (IL6-na), TNF monoclonal antibody (infliximab; IFX), or recombinant mouse IL-1 receptor antagonist (IL1-ra). Hippocampal cell death was evaluated using Fluoro-JadeC as described above. IL1-ra and IFX decreased hippocampal cell death compared to aCSF treatment among CA-dLAN ($p < 0.05$; Fig. 3b), producing levels of neuronal death that were similar to CA-LD mice treated with the vehicle ($p>0.05$). In contrast, treatment with IL6-na did not ameliorate hippocampal neuronal damage associated with CA-dLAN.

A similar pattern was apparent for microglial activation, which is often used as an index of neuroinflammation (*43*). Brain tissue was labeled with Iba-1, an antibody directed against microglia; increased Iba-1 surface area suggests microglial activation (*44*). Significantly greater microglial activation in the CA1, CA2, and CA3 subfields of the hippocampus was apparent among the CA-dLAN mice treated with the vehicle (aCSF) relative to the CA-LD mice treated with the vehicle ($p < 0.05$; Fig. 3c-i). Furthermore, treatment of CA-dLAN mice with IL1-ra or IFX reduced microglia activation in the CA1, CA2, and CA3 ($p < 0.05$; Fig. 3c-i) relative to the CA-dLAN mice treated with vehicle. Iba1 expression in CA-dLAN mice treated with IL1-ra or IFX did not differ from CA-LD mice treated with the vehicle in any of the hippocampal subfields quantified. In contrast, CA-dLAN mice treated with IL-6na had levels of microglial activation in the CA1 that were comparable to CA-dLAN mice treated with the vehicle, while levels of microglial activation in the CA2 and CA3 regions were intermediate between vehicle treated CA-dLAN and CA-LD mice. Thus, inhibiting IL-1 and TNF-α signaling in CA-dLAN mice normalized the microglial and neurodegenerative responses. Furthermore, the results suggest IL-

1 and TNF- α cytokine pathways may be more involved than IL6 in inducing hippocampal damage.

Alternative spectra of lighting minimize light induced damage

In terms of the circadian system, not all lighting is created equal. The intrinsically photosensitive retinal ganglion cells (ipRGCs) that project to the master circadian pacemaker in the SCN contain melanopsin and are most responsive to the blue region of the visible light spectrum ranging from 450 to 485 nm. These wavelengths are present in broad spectrum white light such as natural sunlight and the majority of indoor lighting. Longer wavelengths of lighting do not activate ipRGCs and therefore minimally influence the circadian system (*45, 46*). Thus, we hypothesized that mice exposed to red light at night would more closely resemble the LD phenotype than the dLAN phenotype. Following CA, mice were placed in LD, dLAN [14 h light (150 lux): 10 h dim light (5 lux; 6500K cool white light- containing blue wavelengths)] or a bright-dim red light cycle [rLAN; 14 h light (150 lux): 10 h dim red (5 lux; 636 nm)]. Tissue was collected seven days later, for analysis of neuronal damage via Fluoro-JadeC and microglial activation via Iba-1, as described above.

Unlike full spectrum light at night, dim red light at night did not increase mortality following CA. There were no differences in mortality between CA mice exposed to rLAN versus LD ($p > 0.05$; Fig. 4a). As in the first experiment, CA-dLAN increased hippocampal cell death compared to CA-LD ($p < 0.05$; Fig. 4b, d-f). In contrast, rLAN did not exacerbate ischemic cell death. Hippocampal neuronal damage among CA-rLAN mice resembled that of CA-LD mice ($p > 0.05$) and there was significantly less damage among rLAN mice compared to dLAN mice following CA ($p < 0.05$; Fig. 4b, d-f). We similarly replicated our findings that CA-dLAN exhibited increased microglia activation in multiple hippocampal subfields compared to CA-LD

conspecifics ($p < 0.05$; Fig. 4c, g-i). In contrast, rLAN did not increase post-ischemic microglia activation relative to CA-LD ($p > 0.05$; Fig. 4c, g-i). These results indicate that standard indoor lighting may potentiate neuroinflammation and neuronal damage following CA, whereas alternative lighting using wavelengths greater than ~600nm does not produce the same detrimental biological responses.

Discussion

Cardiovascular disease is the leading causes of death in the US (*47*). The survival rate for cardiac arrest is very low, and the majority of patients who survive live with extensive physical, cognitive, and affective disabilities (*48-50*). However, our murine results indicate that adjusting environmental lighting could prove to be an inexpensive and effective way to improve patient outcome in cardiac intensive care units. Because of patient safety concerns and the need for monitoring, hospital ICU rooms are rarely completely dark and it is not uncommon for patients to be exposed to bright lights (100 lux) several times per night (Figure 1; (*7*)). Indeed, even patients whose eyelids are closed may be affected by the light intrusion (*51*). Here we show that exposing mice to as little as 5 lux of dim light at night after resucitation from CA exacerbates neuroinflammation and neuronal damage, and increases short-term mortality four-fold relative to mice that are maintained in a consistent light-dark cycle. The effects on neuronal damage and mortality appear to be mediated by increased neuroinflammation among CA mice exposed to dLAN. Indeed, TNF-α mRNA expression in the hippocampus is elevated as early as 4 h after exposure to dLAN, and by 24 h the CA-dLAN group has significantly greater TNF-α, IL-1β, and IL-6 mRNA expression compared to the CA-LD group (Fig. 2). These pro-inflammatory cytokines are known to contribute to damage after cerebral ischemia (*52*, *53*). Thus, early changes in the inflammatory response caused by light at night may alter the trajectory of

recovery resulting in higher mortality and increased neuronal damage characterized after one week.

Inhibition of TNF-α, IL-1, or IL-6 signaling among CA-dLAN mice produced 7-day survival rates that approximated or exceeded the survival rate for the CA-LD group (Fig. 3a), although only treatment with IL-1ra or IFX significantly reduced microglial activation and neuronal damage relative to the CA-dLAN mice. Thus, two bodies of evidence point to a role of increased inflammatory responses in mediating elevated neuronal damage among the CA-dLAN mice: (1) both proinflammatory cytokine gene expression and neuronal damage were elevated after exposure to dLAN relative to LD and (2) treatment with IL1ra or IFX prevented the exacerbation of neuronal damage and microglial activation observed among vehicle treated CA-dLAN mice.

Although pharmacological intervention clearly abated the detrimental effects of dLAN on CA-induced microglial activation, neuronal damage, and short-term mortality, a far simpler approach to improving CA outcome is to modify the physical qualities of the nighttime light to prevent increased neuroinflammation. For example, night time red light of the same illuminance as the dim white light did not exacerbate CA-induced neuronal damage or microglial activation in mice. Indeed, the CA-rLAN mice did not differ significantly from CA-LD mice in either of these measures (Figure 3). These data are consistent with studies reporting that red light at night does not affect other aspects of physiology and behavior in humans or other animals to the same extent as broad spectrum white light that contains blue wavelengths (*54*). The effects of night time light are likely mediated by the suprachiasmatic nucleus or “master circadian clock”, which receives input from melanopsin containing ipRGCs in the retina. The ipRCGs are activated by blue light (~480nm, found in outdoor and most indoor lighting, especially fluorescent lights), but

are unaffected by long wavelength light, such as red light. The minimal influence of red light compared to white light on CA recovery suggests that light recognition by the circadian system is involved in dim white light induced exacerbation of CA damage.

In sum, the mouse data presented here suggest that exposure to light at night, a common occurrence in hospital rooms, increases short-term mortality and compromises recovery from cerebral ischemia by exacerbating neuroinflammation. Using red lights at night in hospital rooms or having patients wear goggles that filter lower wave length light could be inexpensive solutions that allow visibility without priming the immune system of the patients. If the effects of white light at night are replicated in cardiovascular patients, these results could have important implications for the design of lighting in clinical settings and could apply to a broad number of conditions and medical procedures that involve ischemia and inflammation, such as stroke, cardiovacular artery bypass graft, sickle cell disease, sleep apnea, and organ transplant.

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Figures

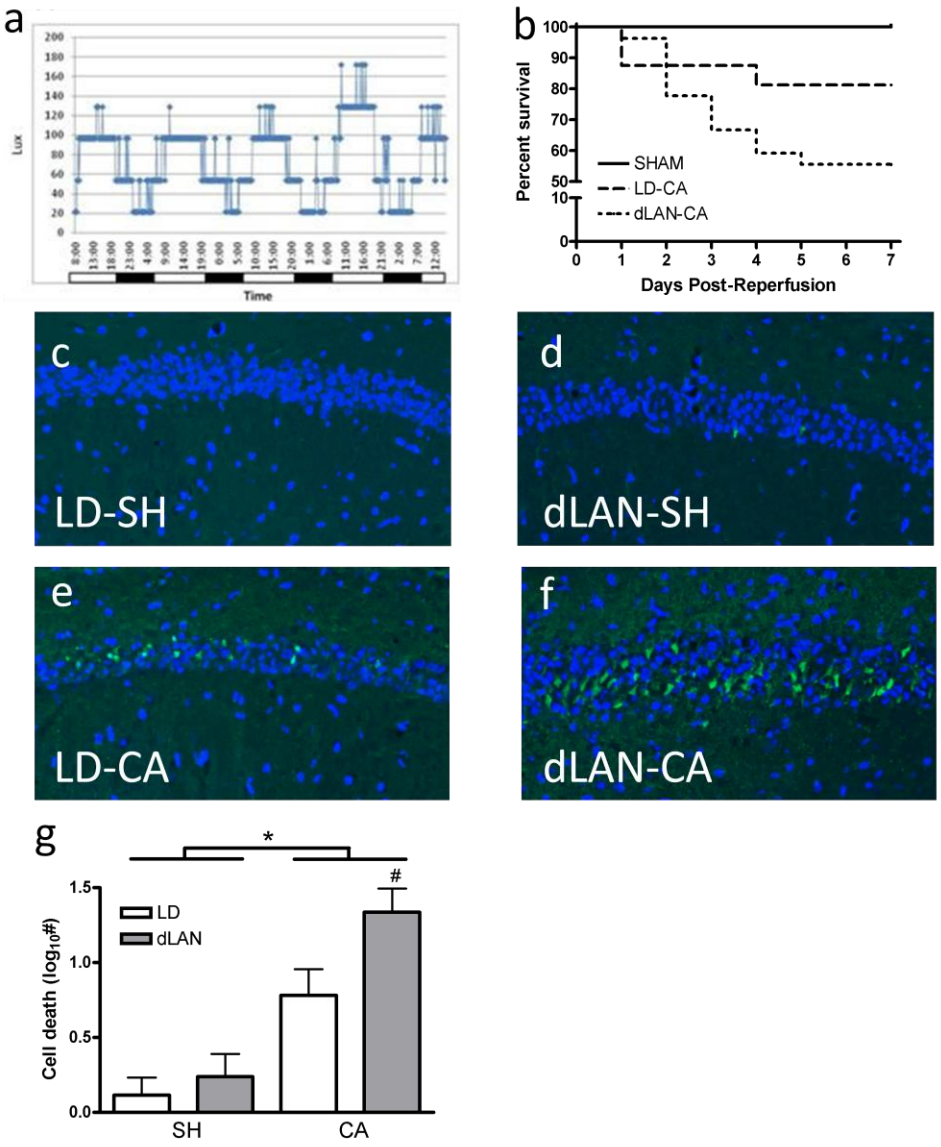


Figure 1. Dim light at night (dLAN) impacts recovery from cardiac arrest and cardiopulmonary resuscitation (CA). (a) Ambient lighting in cardiac intensive care unit patient rooms. (b) dLAN increases mortality following CA in a rodent model. (c-g) dLAN exacerbates CA induced hippocampal cell death as indicated by Fluoro-JadeC staining. Representative Fluoro-JadeC stained sections from the CA1 of (c) LD-SH (d) dLAN-SH (e) LD-CA and (f) dLAN-CA mice.

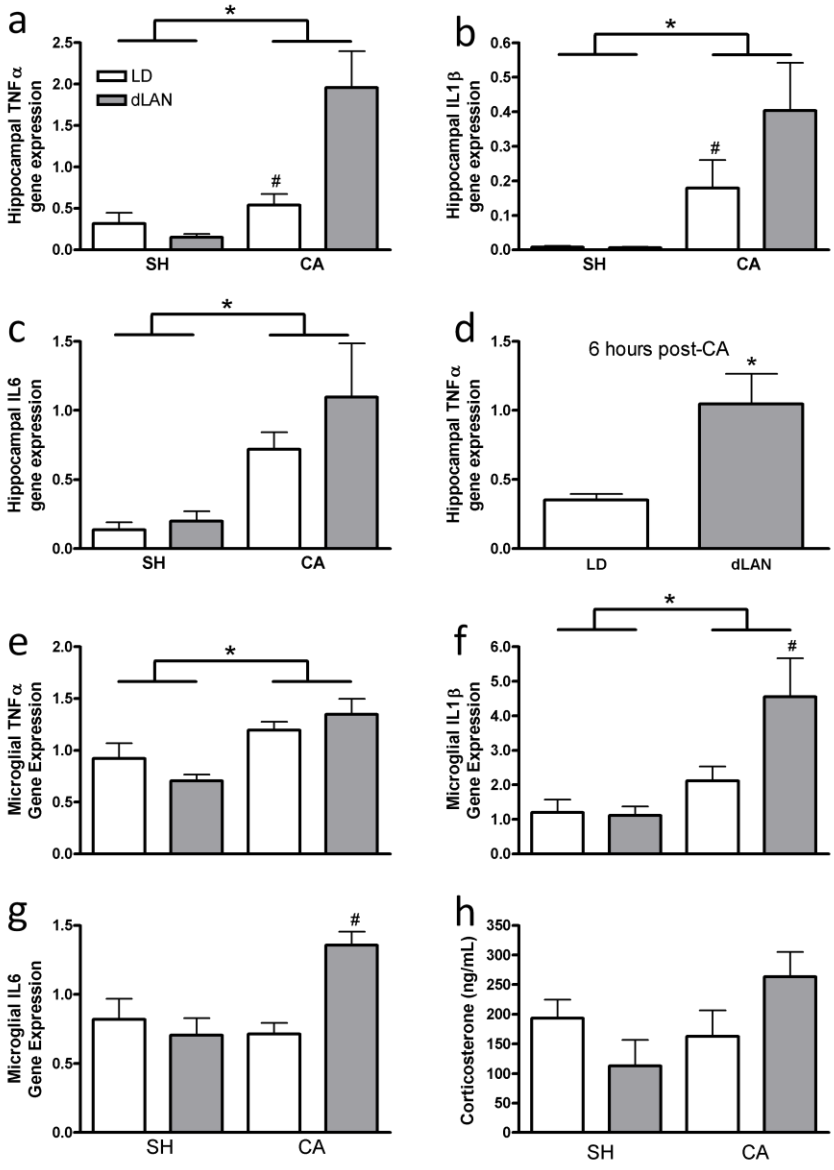
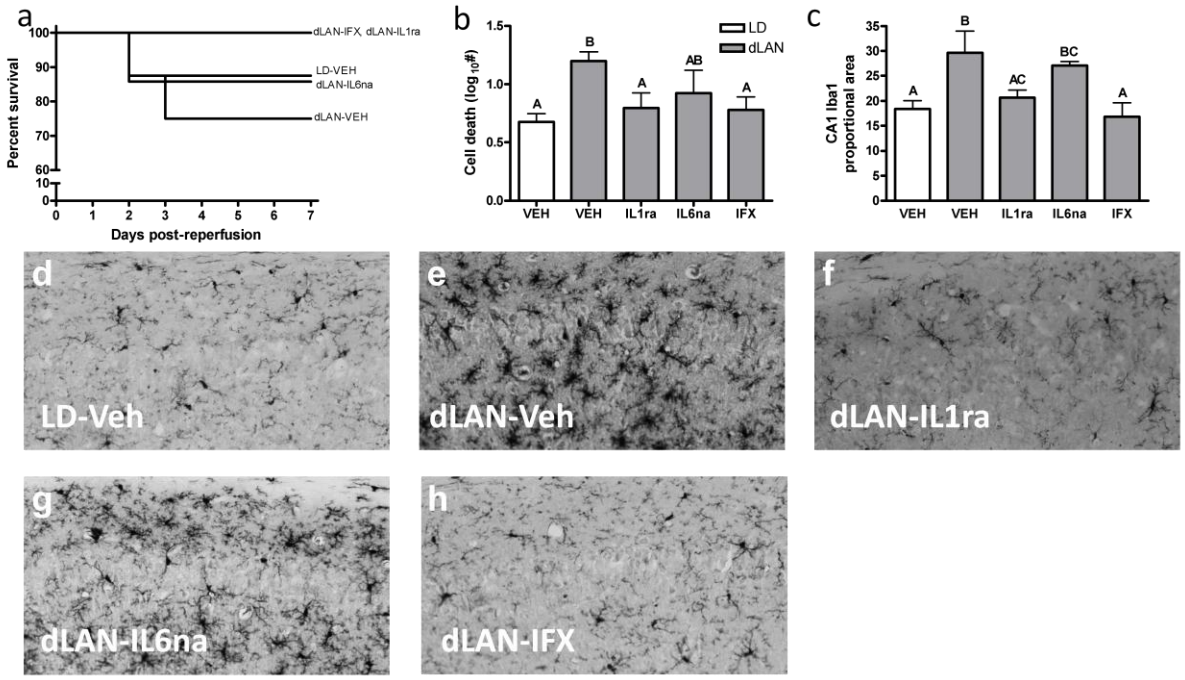


Figure 2. CA induced pro-inflammatory cytokine expression is elevated in the hippocampus and microglia of dLAN mice. Hippocampal (a) TNFα, (b) IL1β, and (c) IL6 gene expression are upregulated 24 hrs following CA and placement in dLAN. (d) TNF-α expression is elevated as early as 6 hours post-CA and only 4 hours of dLAN. Microglial (e) TNFα, (f) IL1β, and (g) IL6 gene expression are also altered 24 hrs following CA and placement in dLAN. (h) Serum corticosterone concentrations are unaffected by light at night.



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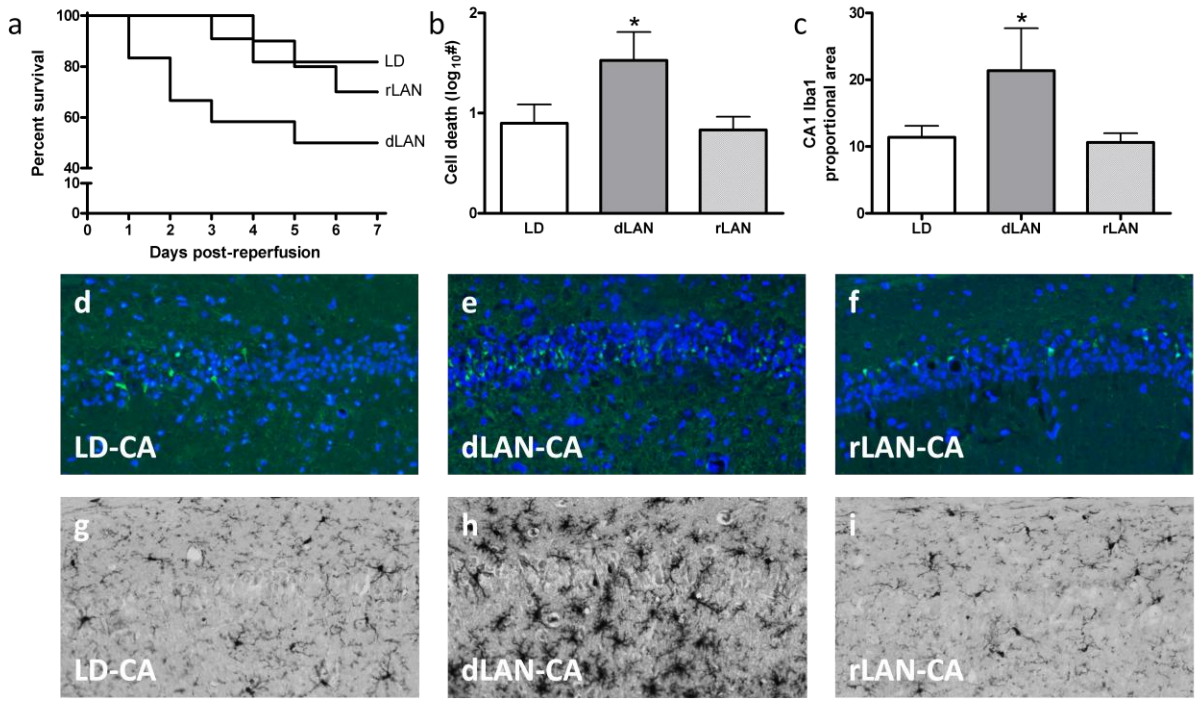
	LD - Veh	dLAN - Veh	dLAN - IL1ra	dLAN - IL6na	dLAN - IFX	p value
CA1	18.3 +/- 1.7	29.6 +/- 4.4	20.6 +/- 1.5	27.1 +/- 0.8	16.822 +/- 2.8	p = 0.005
CA2	15.0 +/- 1.5	24.7 +/- 3.8	17.7 +/- 2.1	16.9 +/- 2.0	13.5 +/- 2.3	p = 0.039
CA3	13.5 +/- 1.4	22.2 +/- 2.6	15.4 +/- 2.3	18.1 +/- 1.5	14.6 +/- 2.5	p = 0.049
DG	18.1 +/- 1.4	27.6 +/- 3.2	18.3 +/- 2.4	20.7 +/- 2.2	17.3 +/- 5.3	p = 0.179
Sub	17.0 +/- 1.5	28.6 +/- 4.1	21.7 +/- 2.9	21.5 +/- 2.5	17.7 +/- 4.9	p = 0.123

Figure 3. Selective inhibition of specific pro-inflammatory cytokines blocks inflammation and neuronal cell death following CA and dLAN. (a) Percent survival following CA and treatment with different cytokine inhibitors (b) Neuronal damage in the hippocampus. (c) Proportional area of Iba1 staining in the CA1. Representative photomicrographs from the CA1 of mice treated with (d) LD-Veh (e) dLAN-Veh (f) dLAN-IL1ra (g) dLAN-IL6ab (h) dLAN-IFX. (i) Proportional area of Iba1 staining throughout the hippocampus.



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	LD	dLAN	rLAN	p value
CA1	11.4 +/- 1.7	21.4 +/- 6.3	10.6 +/- 1.4	*p = 0.045 - log
CA2	12.8 +/- 1.4	29.1 +/- 8.2	11.9 +/- 2.7	*p = 0.018 - log
CA3	10.7 +/- 1.3	13.7 +/- 2.4	10.8 +/- 1.7	p = 0.418
DG	11.4 +/- 2.9	25.8 +/- 5.0	11.4 +/- 1.8	*p = 0.009
Sub	11.7 +/- 1.8	16.5 +/- 3.5	9.8 +/- 1.9	p = 0.166

Figure 4. Manipulation of lighting wavelength minimizes light at night induced damage following CA. (a) Percent survival following CA and placement in LD, dLAN, or rLAN. (b) Neuronal damage in the hippocampus. (c) Proportional area of Iba1 staining in the CA1. Representative photomicrographs of Flourojade staining from the CA1 of (d) LD (e) dLAN and (f) rLAN mice. Representative photomicrographs of Iba1 staining from the CA1 of (g) LD (h) dLAN and (i) rLAN mice. (j) Proportional area of Iba1 staining throughout the hippocampus.

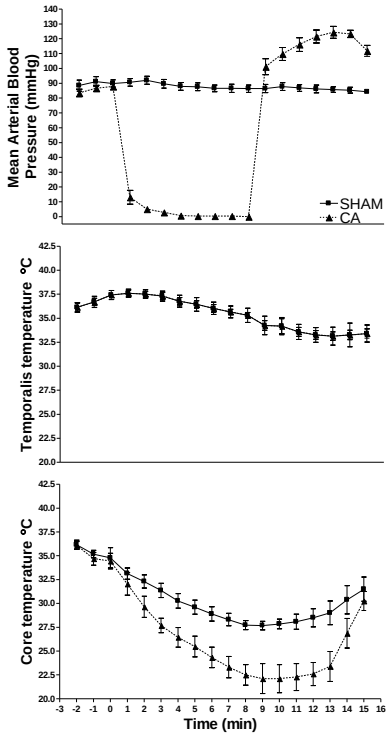

Figure S1. (a) Mean arterial blood pressure (b) head temperature and (c) body temperature during the cardiac arrest and sham procedures.

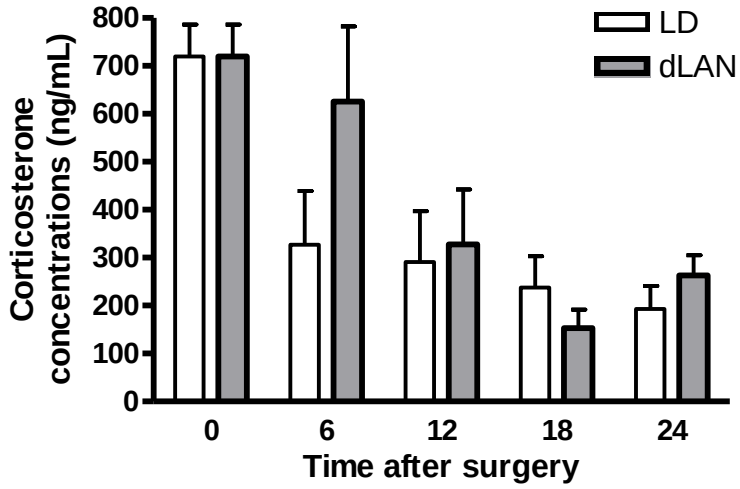

Figure S2. Corticosterone concentrations did not differ between LD and dLAN mice in the 24 h following cardiac arrest.