Effect of environmental lighting on circadian gating of lung injury

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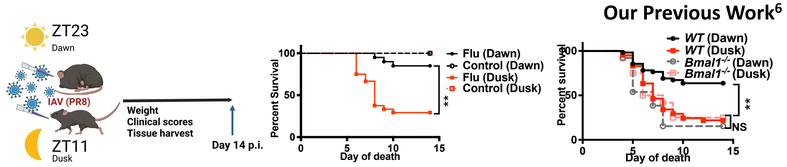
Background

- **While the Suprachiasmatic Nucleus (SCN) in the brain houses the master circadian pacemaker, an endogenous circadian mechanism exists in individual cells.**
- **The circadian clock controls several aspects of host-pathogen interaction.[1, 2, 3, 4]**

Our Previous Work[6]



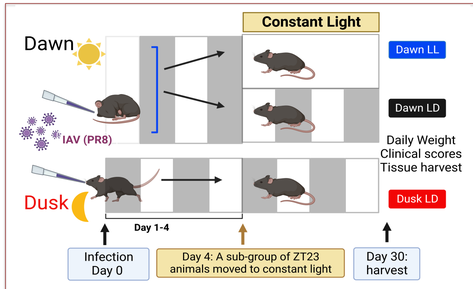
Lights "on": Zeitgeber time "0" (ZT0). ZT23 marks time just before onset of rest (Dawn). ZT11 marks time just before onset of activity (Dusk) since mice are nocturnal.

- **Circadian rhythms provide a time-of-day specific protection from mortality in Influenza A Virus (IAV) infection that is lost in clock-disrupted mice.**
- **Circadian protection from IAV is independent of viral burden and associated with increased inflammation.**

Aim

- **To determine if perturbation to environmental lighting will disrupt lung repair and regeneration**

Experimental Design/Methods

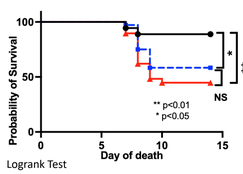


- **Mice: 10-20 weeks old C57bl6/J mice (≈ equal male: female ratio)**
- **Specially designed circadian cabinets: acclimatize mice to reverse light cycling (for the Dawn and Dusk groups)**
- **Locomotor activity (central clock read-out): IR sensors (Actimetrics)**

Infection → **Monitoring weight loss and clinical scores** → **D8 SPC Staining**

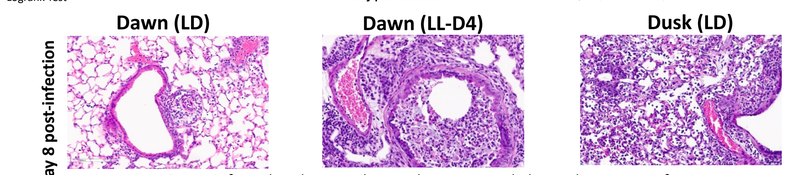
Staining Protocol[7]	Quantification Per Sample
Deparaffinization/Hydration	ImageJ Software (Cell Counter Notice)
Heat Based Antigen Retrieval	~Five 40x Images of Damaged Alveolar Regions
Blocking (5% Donkey Serum)	Manually Count Sftpc⁺ Cells
1° Antibody Addition (SPC 1:100)	Manually Count Nuclei (At least 1000 / lung)
2° Antibody Addition (Alexa-Fluor 488 1:250)	(Total Sftpc⁺ /Total DAPI)*100 = % Sftpc⁺ Cells

Results

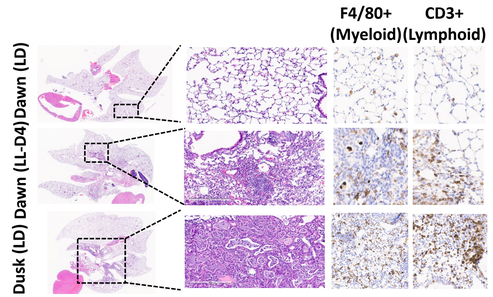


Logrank Test — Mixed-effect Analysis (REML Model)

- **Constant light exposure (after day 4 p.i.) abolishes the clock-driven protection from mortality and morbidity from IAV in the dawn group.**



LL-D4: Group infected at dawn and moved to constant light on day 4 post-infection

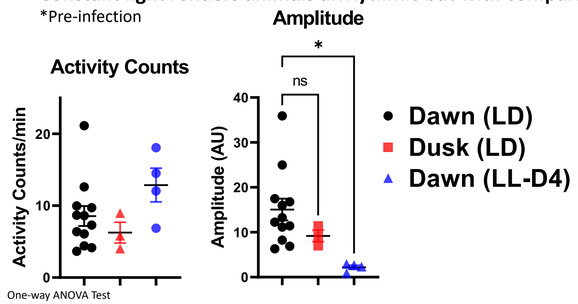


- **Immunopathology worse in LL-D4 and Dusk (LD) groups relative to Dawn (LD).**
- **Lung histology continues to be poor even on Day 30 post-infection.**

Scoring criteria
Peri-bronchial inflammation
Peri-vascular inflammation
Alveolar infiltrates
Epithelial injury

- **Constant light renders animals arrhythmic but with comparable Activity Counts.**

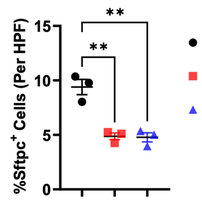
*Pre-infection



One-way ANOVA Test

- **Dawn (LD)**
- **Dusk (LD)**
- **Dawn (LL-D4)**

- **ATII progenitor cells, responsible for restoring lost epithelial cells and regenerating ATI cells, are critical in lung repair and regeneration.[5]**

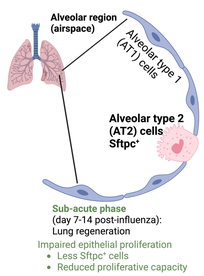


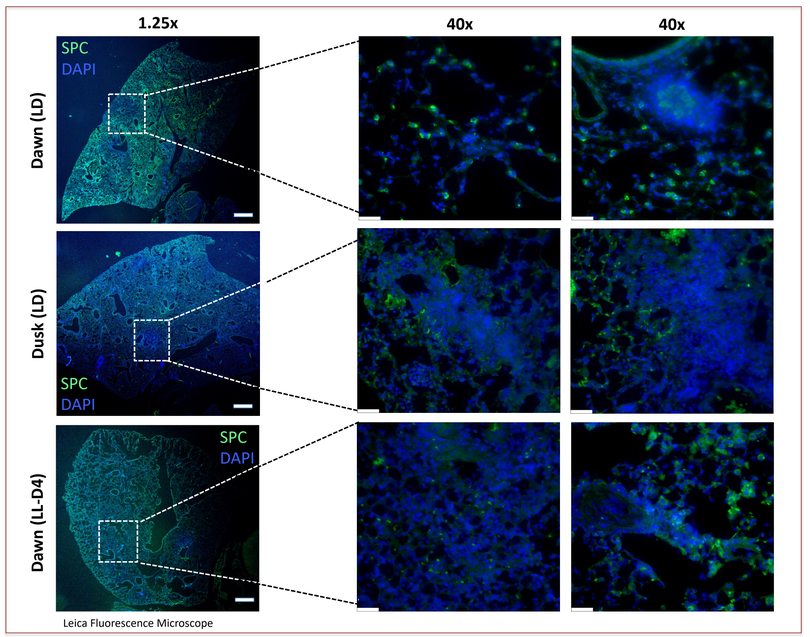
HPF = High-Power Field
*Post-infection

One-way ANOVA Test

- **Dawn (LD)**
- **Dusk (LD)**
- **Dawn (LL-D4)**

- **Quantification of IHC images shows significantly fewer Sftpc⁺ (ATII marker) cells in LL and Dusk groups than in the Dawn group.**
- **Fewer ATII cells indicate poorer lung repair and regeneration potential in the LL and Dusk groups.**

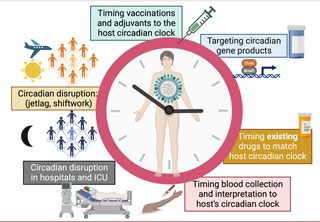




Leica Fluorescence Microscope

Conclusion/Future Directions

- **Disruption of light cycling following influenza infection adversely impacts lung repair and regeneration.**
- **Many implications for clinical care:**
- **Future Direction: Are fewer ATII cells caused by more ATII cell death or less proliferation?**



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References