Inspiratory time (Ti) and expiratory time (Te), while altered by dark phase light stimuli, do not fully explain the impact of these stimuli on respiratory frequency.

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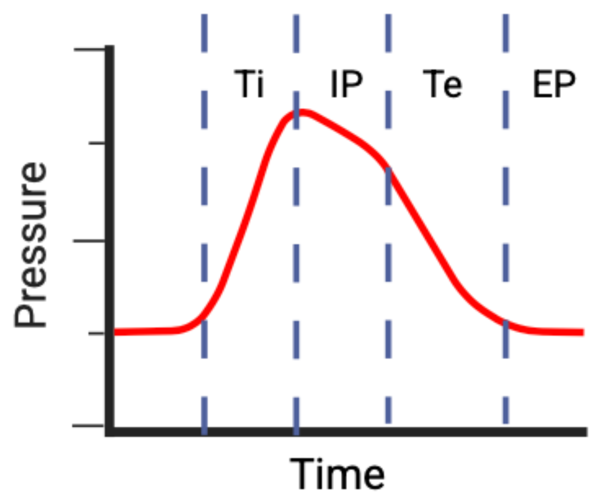
Background

- Respiratory frequency exhibits a daily, 24-hr rhythm in mammals.
- In the absence of light cues, mice are unable to maintain rhythmic frequency, suggesting that a light-driven mechanism regulates breathing independent of endogenous circadian timing (Jones et al., 2021).
- Many behaviors exhibit “negative light masking”, which is defined as a reduction in circadian-driven behavior during an unexpected light stimulus administered in the dark phase.
- Research in our laboratory indicates that the frequency of breathing exhibits negative light masking.
- Frequency can be broken down into four components which are not ofte independently. Two significant determinants of respiratory frequency are inspiratory time (Ti) and expiratory time (Te).
- It is not known whether changes in light-induced respiratory frequency a of changes in one of these four factors which then goes on to affect the others, or if multiple aspects of respiratory frequency are simultaneously affected by light.

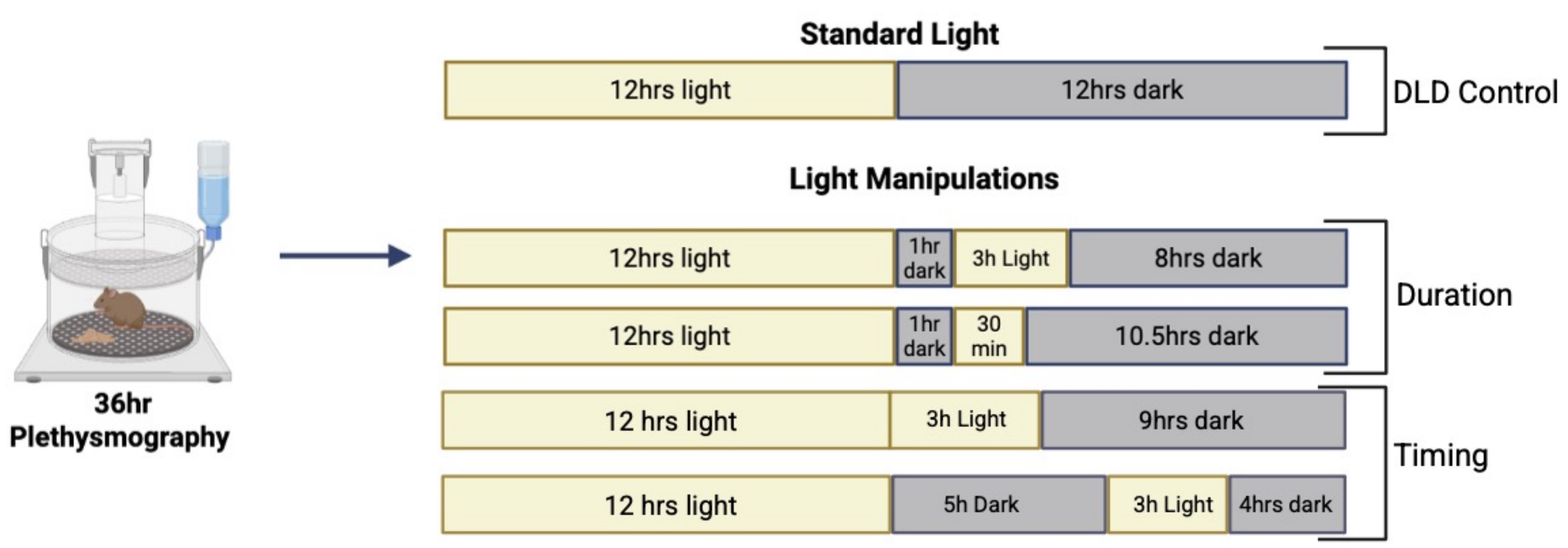


Methods

Animals: Age-matched, male wildtype (C57BL/6J) animals (n = 16) were purchased from the Jackson Laboratory. Mice were housed individually on a standard 12L:12D cycle until experimentation. All studies were approved by the Institutional Animal Care and Use Committee at Marquette University.

Plethysmography: For all experiments, breathing was assessed using whole-body plethysmography. Animals were placed into chambers at ZT6, 6 hours before typical lights off (ZT12). Mice remained in chambers for 36hrs. Threshold requirement settings included a minimum breathing flow rate of 0.5 mL/s, deviation of 80% or less between the inspiratory and expiratory volumes of a putative breath as well as adherence to the following mouse-specific breathing parameters: inspiratory time (60-1000 ms), expiratory time (80-1000 ms), tidal volume (0.04-0.10 mL), and respiratory frequency (10-650 bpm). Food and water were provided *ad libitum* throughout data collection.

Light Conditions: Respiratory data included two 12-hr dark phases. In the second dark phase, mice were exposed to broad-spectrum, white light (~250 lux) for either 3 hours or 30 minutes.

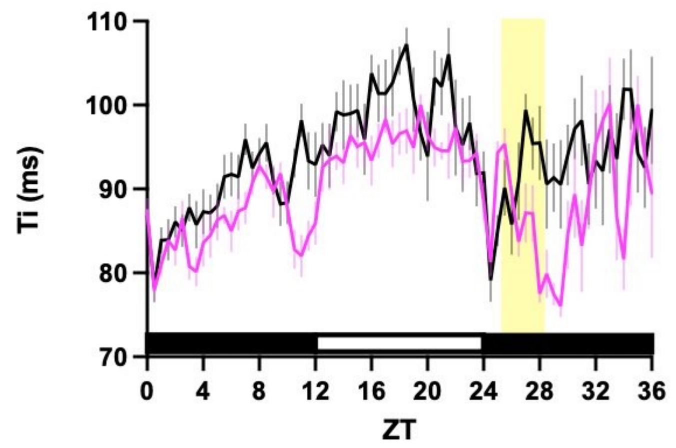


Statistical Analysis: A sliding-window, nonparametric bootstrapping analysis was conducted in R. This approach applied a continuous overlapping window (step size = 1 min, window = 10 min) to assess the temporal consistency of significant effects, as well as the emergence and duration of significant deviations across time. A second nonparametric bootstrapping analysis was also conducted in R. This method compared respiration rates within subjects at parallel time points between a predefined test period and the equivalent control period with and without light manipulation. Statistical significance was determined using bootstrapped confidence intervals, with significance inferred when intervals did not overlap zero (α = 0.05).

Results

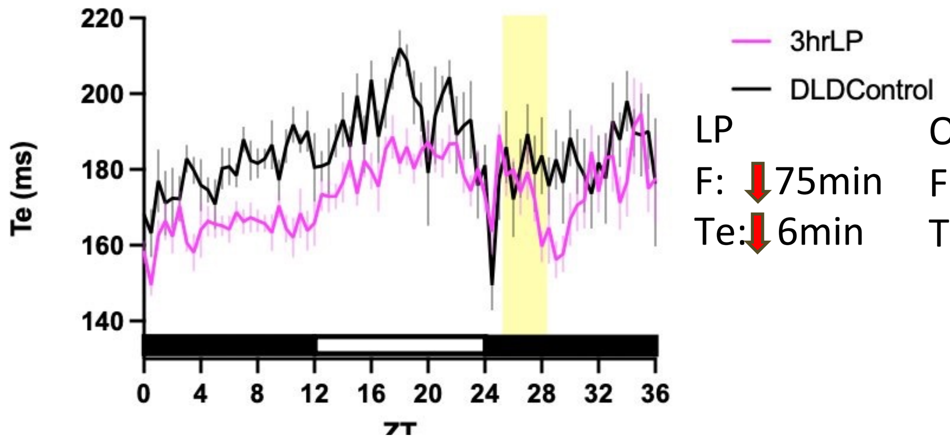
The effect of light stimulus duration on inspiratory time (Ti) and expiratory time (Te).

3hrLP



LP
F: ↓75min
Ti: ↓60min

Offset
F: ↑120min
Ti: ↓80min



LP
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Te: ↓6min

Offset
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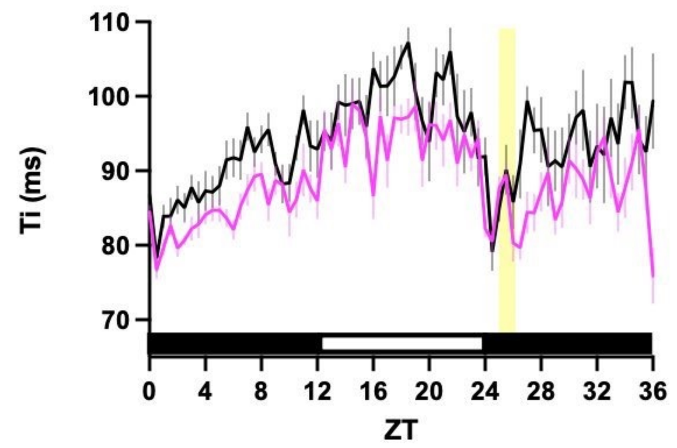
Te: ↓13min

30minLP



LP
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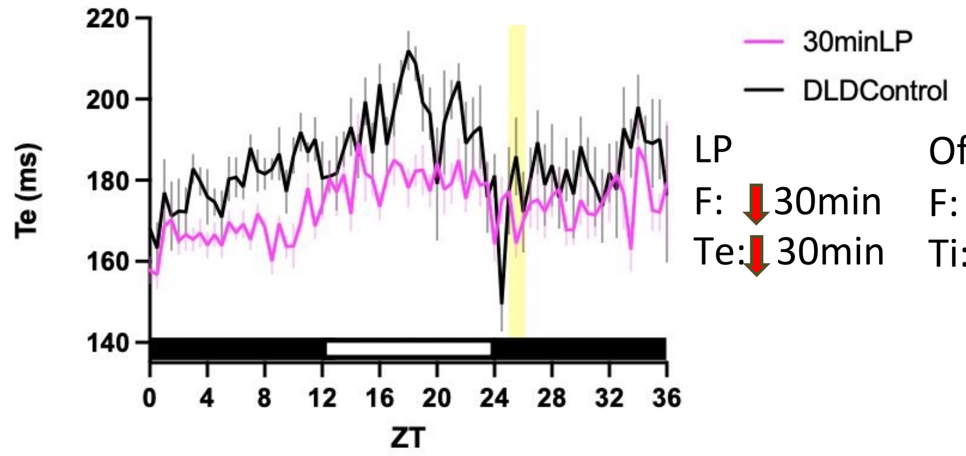
Ti: ↑30min

Offset
F: ↓60min
Ti: ↓60min

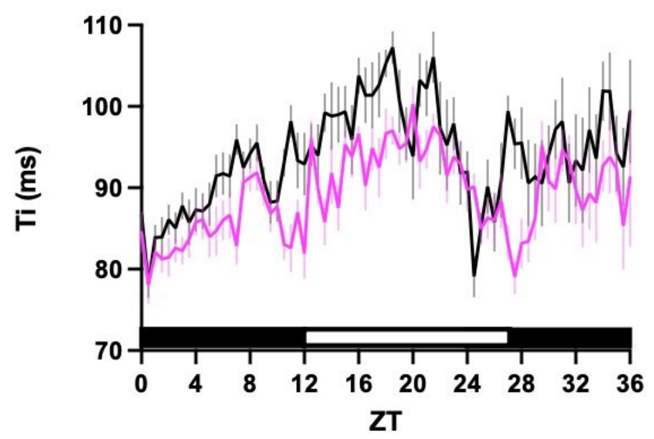


LP
F: ↓30min
Te: ↓30min

Offset
F: ↓60min
Ti: ↓7min

The effect of light stimulus timing on inspiratory time (Ti) and expiratory time (Te).

3hrdelay



LP
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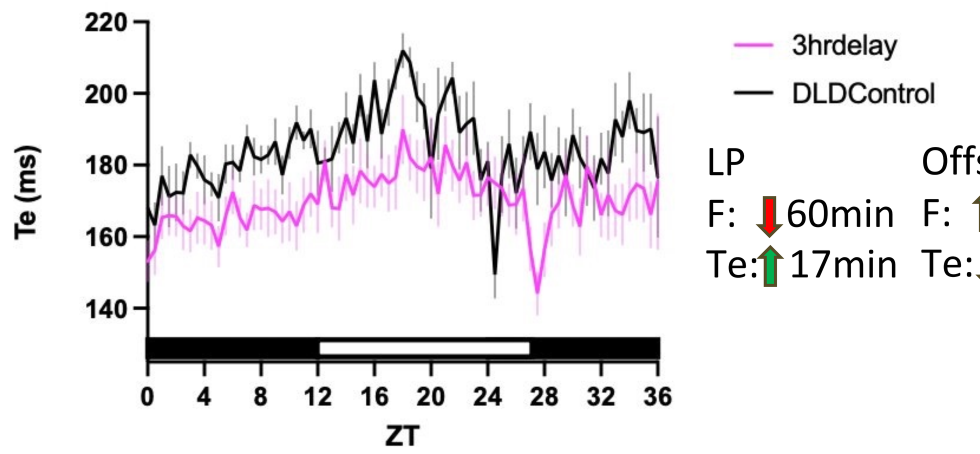
Ti: ↑17min

Offset
F: ↑60min
Ti: ↓60min



LP
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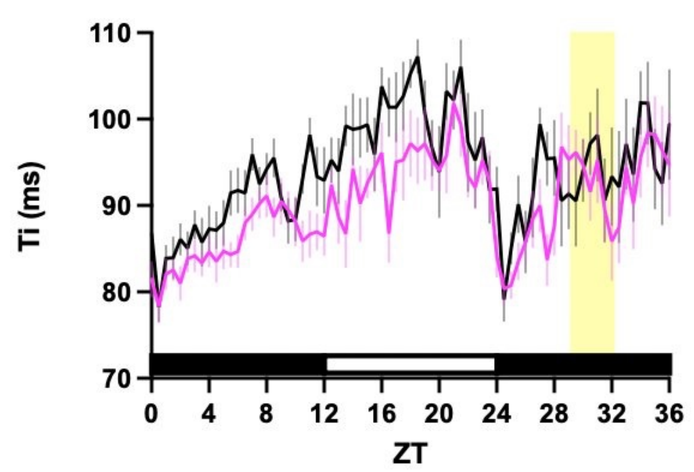
Te: ↑17min

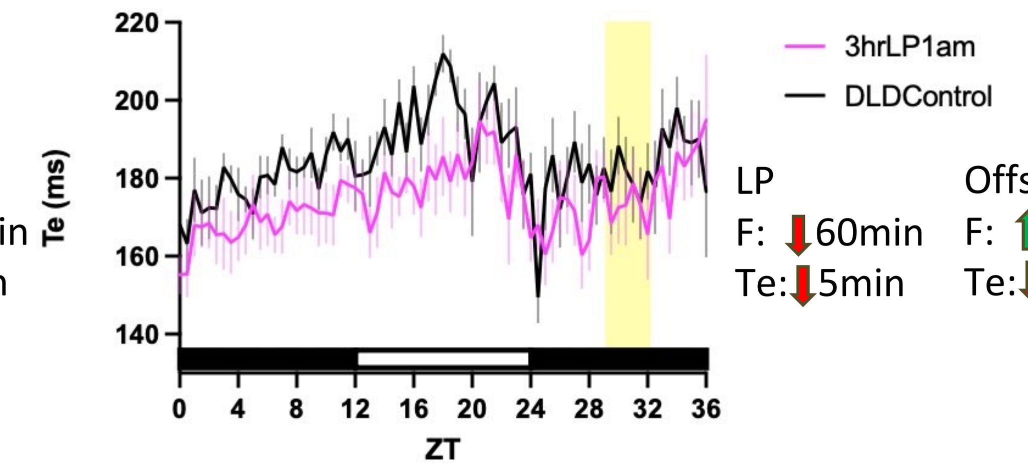
Offset
F: ↑60min
Te: ↓60min

3hrLP1am



LP
F: ↓60min
Ti: ↓180min

Offset
F: ↑120min
Ti: ↓60min



LP
F: ↓60min
Te: ↓5min

Offset
F: ↑120min
Te: ↓5min

Discussion

- Both Ti and Te are significantly altered by varying durations and timings of light exposure during the dark phase.
- Ti displays a consistently more robust effect both during and after light stimuli.
- The relationship between the four elements of frequency is complex and not easily quantified. Here we demonstrate that in some cases, their impact on frequency is additive. We also demonstrate the potential for these parameters to compensate for one another. This complexity underlines the value of examination of these four parameters in conjunction with frequency.
- **Ti** is likely a strong driving factor of changes in respiratory frequency in response to light stimuli of varying **durations.** Variations in the **timing** of these stimuli result in changes in respiratory frequency that are driven by **IP and/or EP.**

Acknowledgements

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