

# NON-PHOTIC CLOCK RESETTING IN MICE

Sarah Tillman & Daniel Janik, University of Wisconsin Eau-Claire



## INTRODUCTION

The circadian clock regulates many aspects of body function including the daily rest/activity cycle and sleep/wake cycle. To stay in synchrony with the environmental light/dark cycle the clock must be reset periodically and environmental light itself is the main stimulus that achieves this resetting. However, other stimuli that are nonphotic (non-light) can reset the circadian clock. These stimuli include exercising in a novel wheel, gentle handling by a human experimenter and other behavioral manipulations. The critical feature of these stimuli that results in resetting is not clear, but recent (unpublished) work suggests that arousal or mild stress may be an important factor.

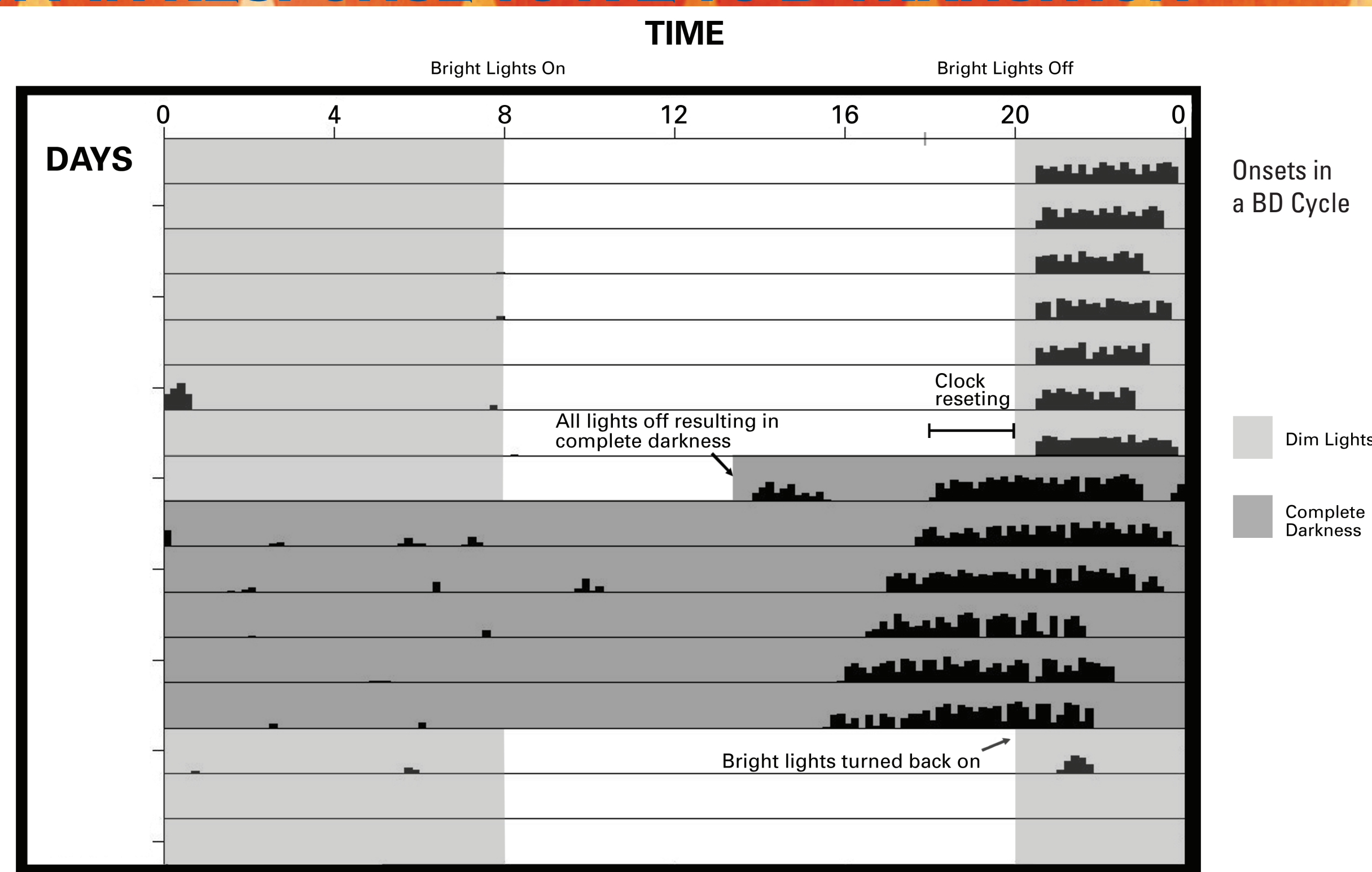
Large discrete shifts of circadian rhythms induced by nonphotic stimulation thus far have been found only in the Syrian hamster. Unlike other species that have been examined (such as rats and mice) only hamsters show sustained physical activity when presented with a novel wheel stimulus. However, a recent study has shown that when hamster circadian rhythms are entrained to a daily cycle of bright light and dim light (as opposed to bright light and darkness), their rhythm shifts when they undergo a single abrupt transition to continuous darkness in the middle of the bright phase. Interestingly, many hamsters that reset in response to this stimulus do not appear to engage in much or any physical activity.

We decided to test whether mice would show clock resetting under these conditions since apparently no sustained physical activity is required. We also asked whether any observed phase shifts could be blocked by propranolol administration, as they are in hamsters.

## METHOD

- Animals and setting:**
  - C57 Black mice were held individually in bucket cages with standard running wheel.
  - The light cycle (BD) was 12 h of bright light (300 lux) and 12 h of dim light (levels < 0.5 lux)
- Circadian Activity Measurements**
  - Activity was recorded using magnetic switches attached to the running wheels and registered continuously by computer.
  - Mice were held in bright-dim light (BD) for at least 11 days.
  - Clock-resetting was assessed by releasing the animals into constant darkness for 3-4 days.
  - Linear regression analysis was conducted prior to the light-to-dark (L-to-D) transition and after the L-to-D transition to determine baseline and post-pulse activity onsets.
  - Phase shifts were calculated by taking the difference between the two onset times.
- Experiment 1: Do mice show a phase-specific clock resetting in response to a L-to-D transition?**
  - 13 male mice assigned to 6 groups in a counter-balanced design
  - After baseline activity onsets were determined, animals were released into constant darkness at zeitgeber time (ZT) 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24/0. ZT 12 is the time of the daily bright-to-dim transition. Therefore ZT 0-12 is the animals' day and ZT 12-24 is the animals' night.
- Experiment 2: Is the observed change in activity onsets due to unmasking? Is it specific to a transition to constant dark?**
  - Nine mice
  - After a minimum of 11 days in BD, mice were treated in one of 3 ways
    - Transition to constant dim at Zt 6
    - Transition to constant dark at Zt 12
    - Transition to constant dim at Zt 12
- Experiment 3: Does propranolol reduce clock-resetting induced by L-to-D transitions?**
  - Propranolol (a beta-adrenergic antagonist) blocks the action of noradrenaline, a neurochemical involved in arousal and stress responses. It has been shown to reduce clock-resetting in the hamster.
  - 10 male and female mice assigned to 4 groups in a counter-balanced design
  - Injected animals subcutaneously with propranolol at doses of 0 (control), 5, 10, and 20 mg/kg immediately before L-to-D transition at ZT 6.
  - Data are preliminary, experiment is not yet complete.

## INDIVIDUAL RECORDING OF A MOUSE'S ACTIVITY IN RESPONSE TO A L-TO-D TRANSITION

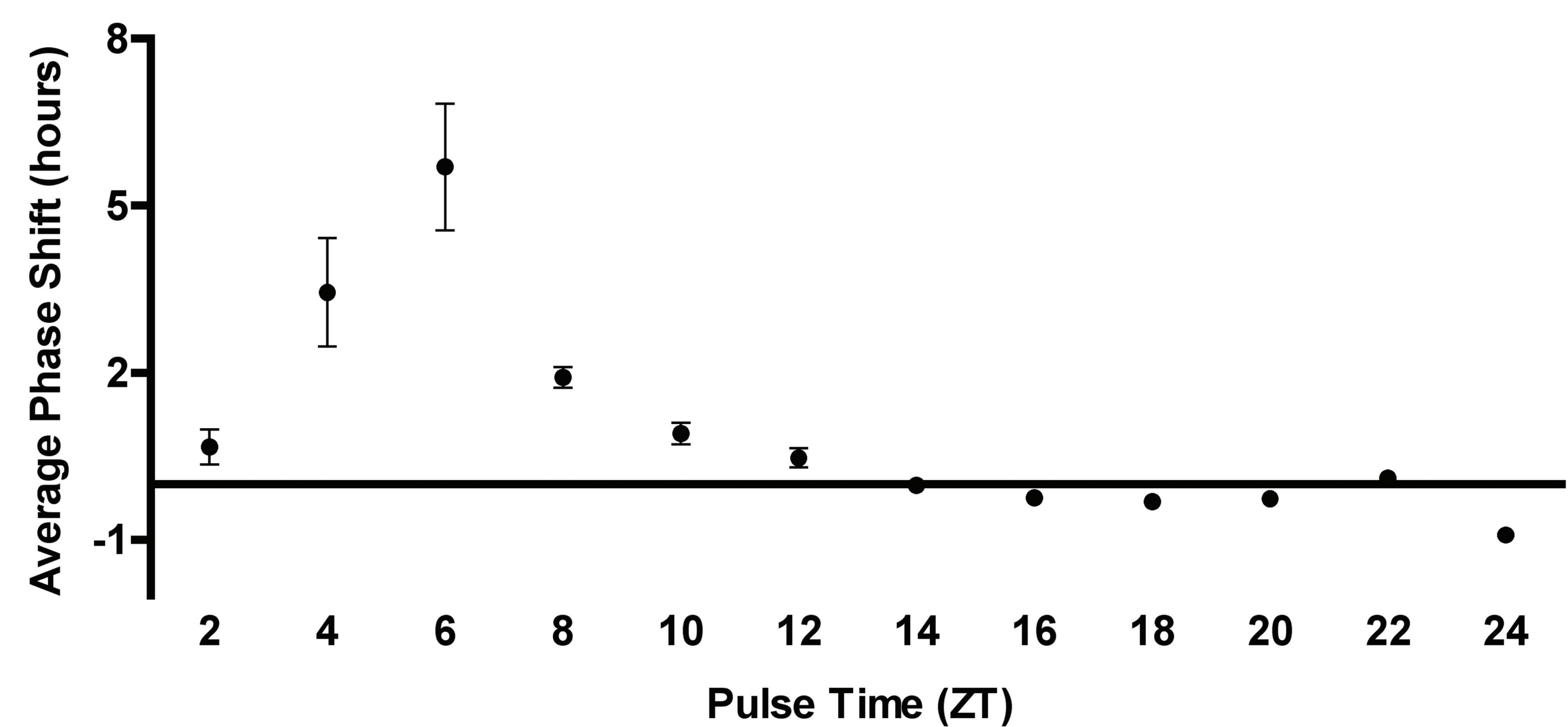


## CONCLUSIONS

- This study shows, for the first time, that mice display discrete clock-resetting to nonphotic stimulation.**
  - This bolsters the notion that nonphotic clock-resetting is not idiosyncratic of hamsters; it may be widespread among mammals and other vertebrates.
  - Because the mouse is a major biomedicine model organism, the genetic and other tools developed for the mouse can be brought to use on the problem of nonphotic clock-resetting.
- Nonphotic clock-resetting in the mouse appears to have the same underlying basis as that shown in the hamster.**
  - Shape of the PRC is similar
  - Phase shifts are suppressed by propranolol.

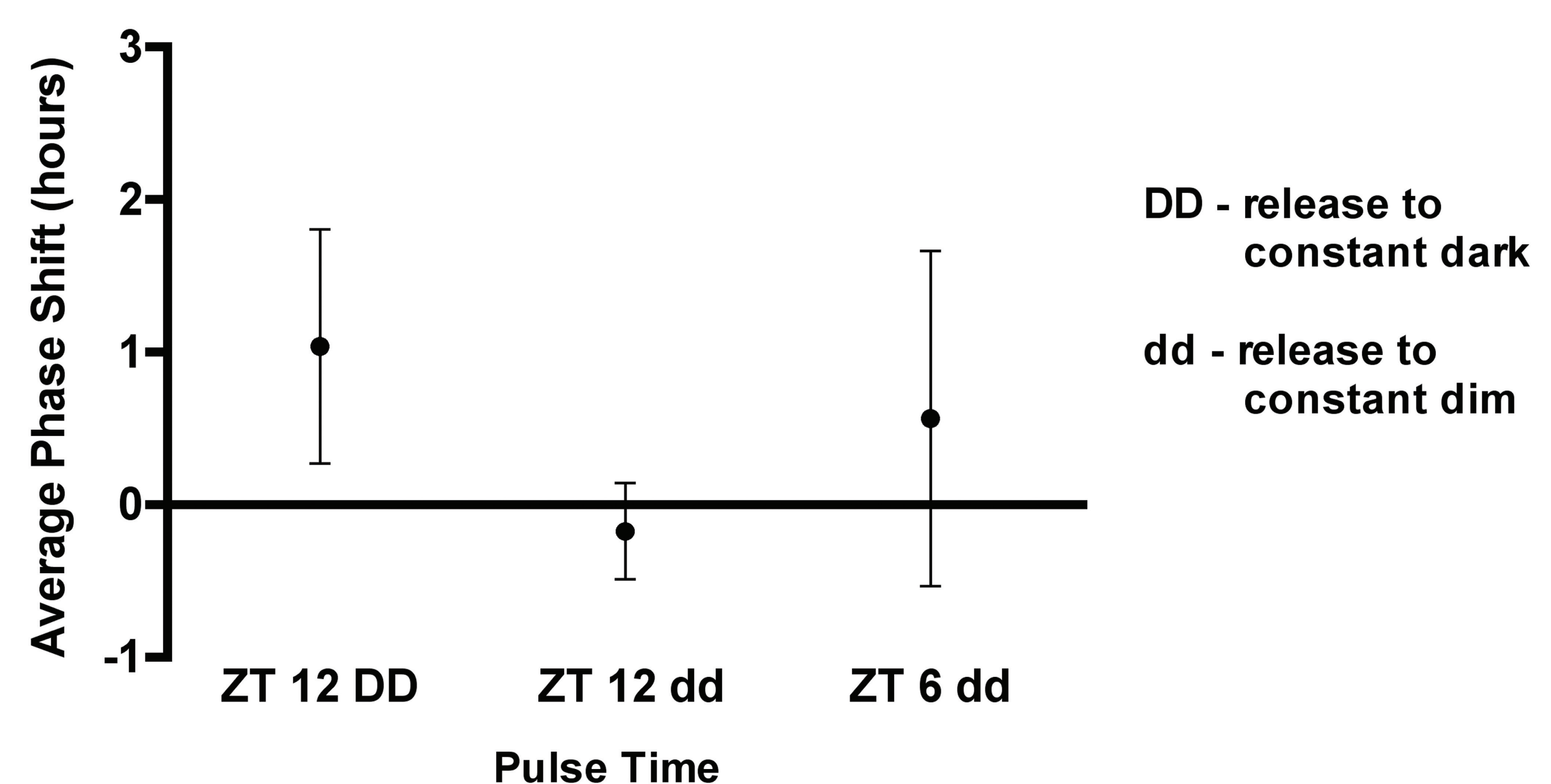
## RESULTS

- Phase Response Curve**
  - Mice showed phase-specific clock-resetting with peak responses at ZT 6 and little resetting during the night (ZT 12-24).
  - The mice PRC is generally consistent with the hamster PRC



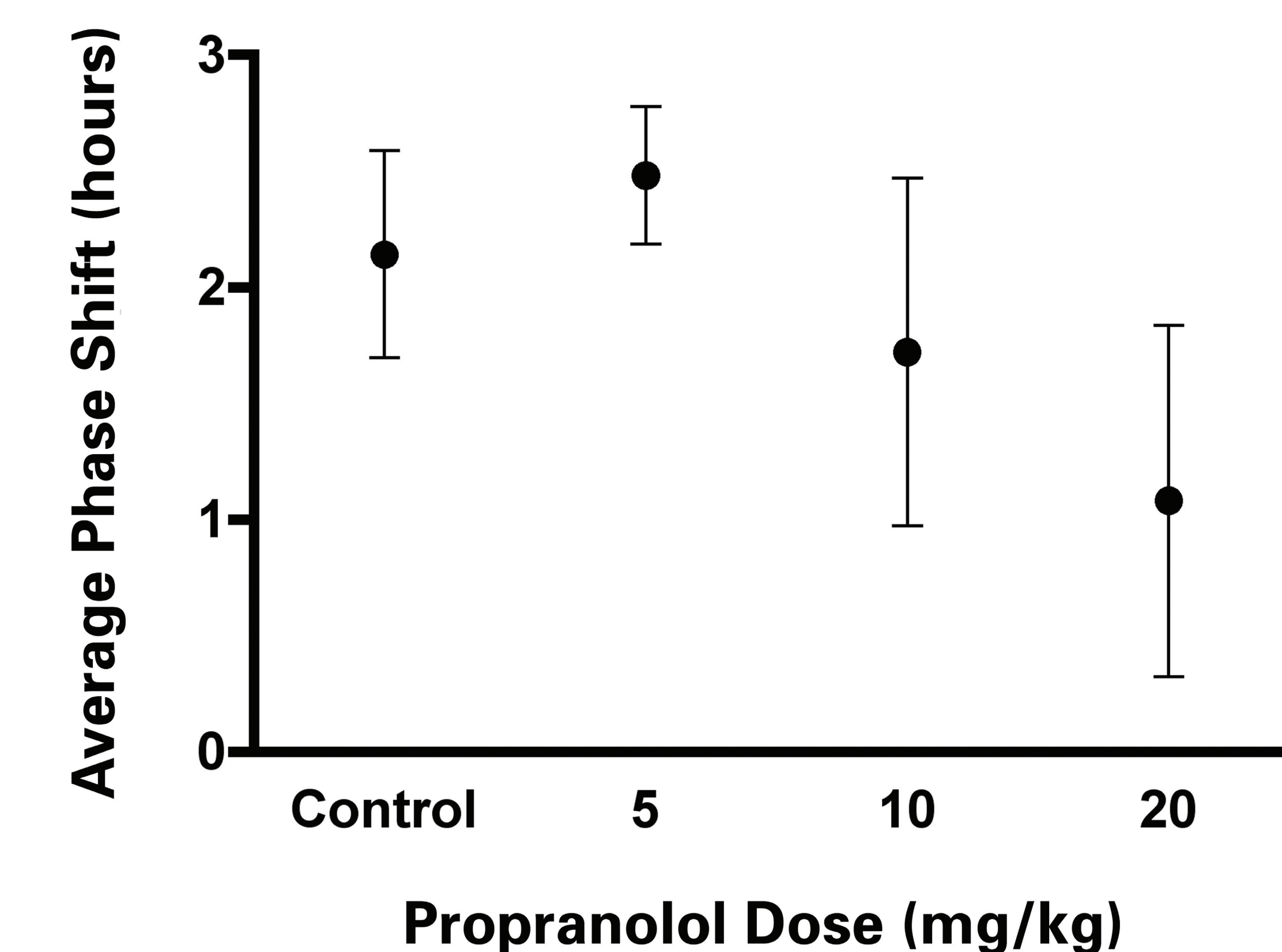
- Stimulus Specificity: Bright-to-dim transitions at ZT 6 and ZT 12 reduce minimal resetting, as do light-to-dark transitions at ZT 12. Therefore:**

- Observed phase shifts are not the result of unmasking (lifting of the direct inhibitory effect of light on activity)
- Complete darkness is necessary to induce phase shifts.



- Suppression of Clock-Resetting by Propranolol**

- Propranolol appears to suppress clock resetting just as it does in the hamster.
- Results are preliminary; more data need to be gathered.



## ACKNOWLEDGMENTS

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