Sympathetic Activation and Circadian Clock Resetting in Mice

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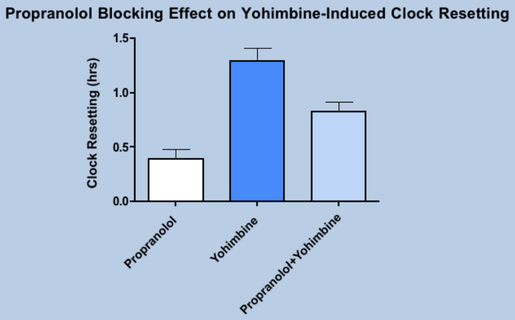
Introduction

Mice are nocturnal animals that exhibit predictable activity patterns controlled by their internal (circadian) clock. We have demonstrated that a mouse's circadian clock can be reset 1-3 hours earlier by various manipulations: transfer from continuous light to complete darkness (BD light cycle), administration of yohimbine, and administration of methamphetamine. We wanted to know if any commonality exists among these treatments that might result in clock resetting.

First, we showed that the phase-shifting effects of yohimbine can be blocked when given in combination with the beta-adrenergic antagonist, propranolol. We also observed increased locomotion during clock-resetting experiments, possibly as a result of mild stress and sympathetic activation. One common sign of sympathetic activation is increased defecation. Therefore, we expected to see an increase in defecation for animals treated with each manipulation, indicating that the treatments were activating the sympathetic nervous system.

Effect of Propranolol on Yohimbine Clock-Resetting

Animals were entrained in 12 hrs light: 12 hrs darkness for 14 days. Animals were given a series of 20 mg/kg propranolol or saline and either 5 mg/kg yohimbine or water injections. Lights were turned off immediately following the injection at ZT6 and animals were left in darkness for 3 days.



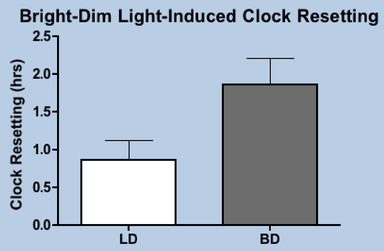
Results

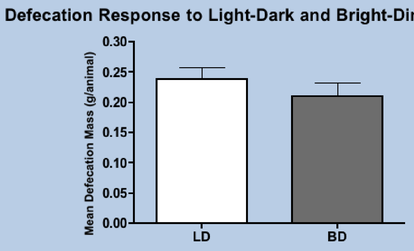
Animals that were given a combination of propranolol and yohimbine injections showed less clock-resetting than animals that were given saline and yohimbine injections ($p<0.001$).

Sympathetic Activation and Clock Resetting

Bright-Dim Light Cycle

Animals were entrained in 12 hrs light: 12 hrs dim light for a minimum of 14 days. Animals were placed in small holding cages and lights were turned off at ZT6. After three hours in the dark, animals were transferred back to wheel cages and kept in darkness for 3+ days. Fecal output was collected from holding cages and weighed.





Results

Transfer from continuous light (BD) to complete darkness induced greater clock resetting than the LD controls ($p=0.0250$). However, there was no significant difference in the fecal output of the LD and BD-entrained animals ($p=0.3315$).

Acknowledgements

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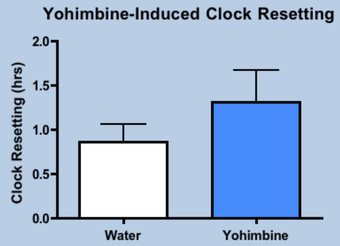
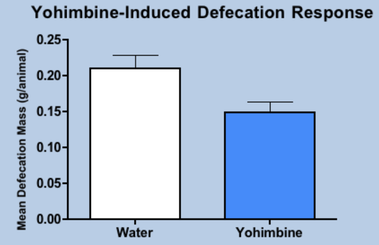
Yohimbine

Animals were entrained in 12 hrs light: 12 hrs darkness for 28 days. A subcutaneous injection of either 5 mg/kg yohimbine or a water control was administered at ZT6. Animals were placed in small holding cages and lights were turned off immediately after the injection. After three hours in the dark, animals were transferred back to wheel cages and kept in darkness for 3+ days. Fecal matter was collected from holding cages and weighed.



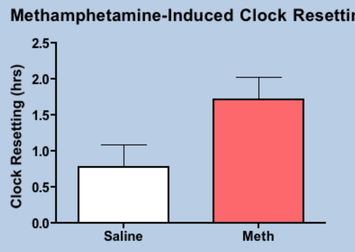


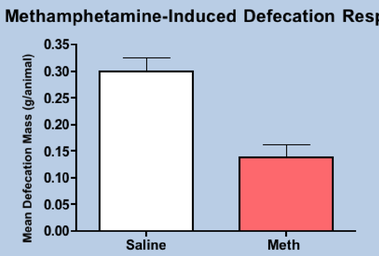
Results

Animals who received a yohimbine injection did not show greater clock resetting than the water control ($p=0.2840$) in this particular trial. However, animals receiving yohimbine showed significantly less fecal output in the first three hours post-treatment than animals receiving a water injection ($p=0.0241$).

Methamphetamine

Animals were entrained in 12 hrs light: 12 hrs darkness for 28 days. A subcutaneous injection of either 40 mg/kg methamphetamine or a saline control (0.9%) was administered at ZT6. Animals were placed in small holding cages and lights were turned off immediately after the injection. After three hours in the dark, animals were transferred back to wheel cages and kept in darkness for 3+ days. Fecal matter was collected from holding cages and weighed.





Results

Animals who received a meth injection showed significantly greater clock resetting than the saline control ($p=0.0493$), with average phase shifts of 1.75 hours. Animals receiving meth showed significantly less fecal output in the first three hours post-treatment than animals receiving saline ($p=0.0005$).

Discussion and Future Research

The literature suggests that fecal output is an acceptable indicator of sympathetic activation. Our results showed that contrary to our hypothesis, animals given clock-resetting agents had lower fecal output in some cases, but never higher fecal output. However, we also noticed that our controls in the clock-resetting experiments showed higher phase shifts while animals treated with the clock-resetting agents showed lower phase shifts than in experiments where fecal output was not measured (for example, see experiment 1).

One possible reason for these discrepancies may have been a slight change in experimental procedure. In earlier experiments (including experiment 1) animals had continuous access to a running wheel after the manipulation. However, in the later experiments in which fecal output was measured, animals were transferred to a holding cage after treatment and did not have access to a running wheel for about 3 hours. We believe that this immediate wheel access may be necessary for the induction of the larger clock-resetting and fecal output. Future research will involve running experiments with the current protocol alongside experiments that do not include the three-hour transfer to a non-wheel cage to determine the importance of the wheel immediately after treatment.