

# Nicotine-induced circadian activity patterns under fixed and variable zeitgebers

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## Introduction

Several addictive drugs are known to entrain endogenous circadian rhythms, including nicotine (1), methamphetamine (2), cocaine (3), and alcohol (4). In rats, administration of these drugs every 24 hours produces both pre-injection (anticipatory) activity and a post-injection (evoked) response. These entrained activity bursts will persist for at least 1 day when injections are withheld and no other timing cues are available. Many of these preclinical studies have used constant lighting conditions and/or restricted feeding to isolate the drug-induced rhythms from the influence of the light- and food-entrainable oscillators. Because human addicts rarely live under such controlled conditions, this study assessed how nicotine-induced circadian rhythms are affected by variations in food and light/dark schedules.

Experiment 1 examined food schedules and Experiment 2 examined light/dark schedules. Nicotine injection times were shifted in the different injection series to examine the rats' ability to re-entrain to new times, and to avoid "marking" of a particular time of day. Based on the results of White et al. (2000), we expected that the different feeding schedules would have little effect on the circadian activity rhythms. Since the change from light to dark is a powerful activating zeitgeber, we expected the different light/dark schedules to affect both pre-injection and post-injection responses.

## Methods

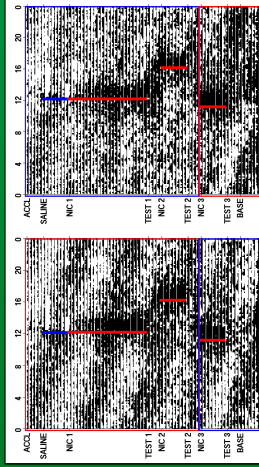
### Experiment 1: Food Schedules

Sixteen female rats were housed for 72 days in individual wheel boxes under constant dim light. The eight rats in group RT-AD initially had feeding rate-limited to no more than two 97-mg pellets every 5 minutes. The remaining 8 rats (group AD-RT) initially were fed *ad libitum*. Upon completion of a 5-day acclimation and an 8-day saline injection series, each rat was administered nicotine at a dosage of 1.0 mg/kg once every 24 hours for 3 injection series, the first lasting 24 days, and the second and third lasting 8 days. Each injection series was followed by a 4-day test phase in which injections were withheld. Prior to injection series 3, the food schedules for each group were switched so group RT-AD was then fed *ad libitum* and group AD-RT was on rate-limited feeding.

### Experiment 2: Light/Dark Schedules

Sixteen female rats were housed for 64 days in individual wheel boxes under rate-limited feeding. Eight rats assigned to group FX-VR were initially under a fixed 10L:14D schedule. The remaining 8 rats (group VR-FX) initially were under a variable L/D schedule, in which a 10-hour light period was presented at a random time each day. The study schedule and injections were identical to Experiment 1, except nicotine injection series 1 lasted only 16 days. Prior to the beginning of injection series 3, the light/dark schedules for each group were switched so group FX-VR was then on the variable schedule and group VR-FX was on the fixed 10L:14D schedule.

## Experiment 1: Food Schedules

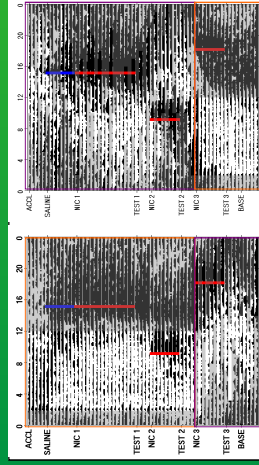


Group RT-AD

Group AD-RT

Figure 1. Wheel running actograms under rate-limited (red outline) and *ad libitum* (blue outline) feeding for the two study groups. Daily nicotine injection times are marked with vertical red lines, and saline injection times are marked with vertical blue lines. Under both feeding schedules, wheel running entrained to the nicotine injection time and persisted around this time when injections were withheld on test days.

## Experiment 2: Light/Dark Schedules



Group FX-VR

Group VR-FX

Figure 4. Wheel running actograms under fixed 10L:14D (orange outline) and variable 10L:VarD (purple outline) light schedules for the two study groups. Daily nicotine injection times are marked with vertical red lines, and saline injection times are marked with vertical blue lines. Horizontal gray bars mark the dark phase of the cycle. Under both light/dark schedules, wheel running entrained to the nicotine injection time and persisted around this time when injections were withheld on test days.

## Results

### Experiment 1: Food Schedules

Wheel running, feeding, and drinking entrained to the nicotine injection times under both rate-limited and *ad libitum* feeding schedules. Typical pre-injection and post-injection responses were observed in both groups. On the test days, entrained activity persisted for 1-2 days around the injection time. When the nicotine injection time was shifted in series 2 and 3, activity re-entrained to the new injection times. Overall, there were no significant differences in nicotine-induced rhythms between the rate-limited and *ad libitum* food schedules.

### Experiment 2: Light/Dark Schedules

Wheel running, feeding, and drinking entrained to nicotine injection times under both fixed and variable light/dark schedules, although some dark-elicited feeding and drinking was also observed. On the test days, entrained activity persisted for 3-4 days around the injection time. When the nicotine injection time was shifted in series 2 and 3, activity re-entrained to the new injection times. While there was no significant difference in the pre-injection activity response, post-injection activity was extended by approximately 4 hours under the fixed light/dark schedule.

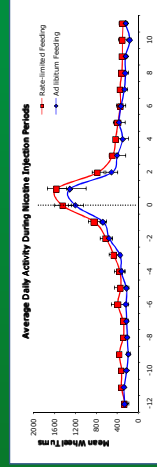


Figure 2. Mean wheel running in one-hour bins under rate-limited (red line) and *ad libitum* (blue line) feeding. The nicotine injection was administered at time 0, indicated with the dotted black line. There was no significant difference in overall wheel running between the two feeding schedules.

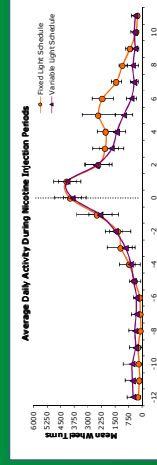


Figure 5. Mean wheel running in one-hour bins under fixed 10L:14D (orange line) and variable 10L:VarD (purple line) light schedules. The nicotine injection was administered at time 0, indicated with the dotted black line. The post-injection wheel running response was extended by approximately 4 hours under the fixed light/dark schedule.

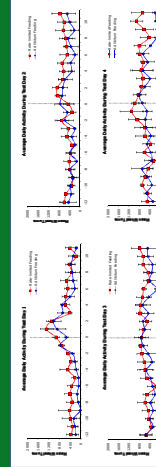


Figure 3. Mean wheel running in one-hour bins under rate-limited (red line) and *ad libitum* (blue line) feeding on the test days when no injections were administered. The dotted black line indicates the previous nicotine injection time. Under both feeding schedules, wheel running persisted around the injection time for at least 1 test day.

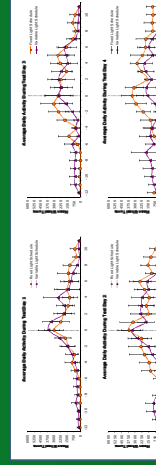


Figure 6. Mean wheel running in one-hour bins under fixed 10L:14D (orange line) and variable 10L:VarD (purple line) light schedules on the test days when no injections were administered. The dotted black line indicates the nicotine injection time. Under both feeding schedules, wheel running persisted around the injection time for 3-4 test days.

## Conclusions

In short, nicotine-entrained circadian activity rhythms were not affected by food availability. However, fixed light/dark schedules extended the post-injection activity effect, most likely as a function of the dark part of the cycle. Because the nicotine-entrained rhythms were not eliminated by either the endogenous light- or food-entrained activity rhythms, these results provide evidence that the entraining effect of the daily administration of addictive drugs occurs independently of the light- and food-entrainable oscillators.

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