

# PRE- AND POST-NICOTINE CIRCADIAN ACTIVITY RHYTHMS ARE DIFFERENTIATED BY A PAIRED ENVIRONMENTAL CUE

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## Introduction:

Nicotine is a highly addictive psychomotor stimulant (Hakan & Ksir, 1988). Pavlovian pairings of nicotine with an environmental cue are known to play a role in the addiction process. Stimuli paired with drug effects eventually become capable of eliciting "craving" behavior in the absence of the drug (Siegel, 1999).

Previous studies have shown that circadian rhythms entrained to drug effects may also play a role in addiction. For example, rats anticipate daily methamphetamine injections in the same way they anticipate daily meals, with increases in locomotor activity (Kosobud et al., 1998, Pecoraro et al., 2000). Post-injection ambulatory activity has also been shown to vary with the time of daily nicotine injections (Kita et al., 1986).

This study investigated the relationship between conditioned stimuli paired with nicotine effects and the circadian rhythms associated with nicotine injections.

## Methods:

Sixteen female rats were housed individually in wheels and attached cages with food and water for 56 days under constant low light. Food access was rate-limited to no more than two 97-mg pellets every five minutes to eliminate the potential activity entraining effects of large meals (White et al., 1999). All rats underwent 3 nicotine injection phases of 7-14 days. Each phase was followed by a 3-4 day test period to determine which events entrained activity bursts.

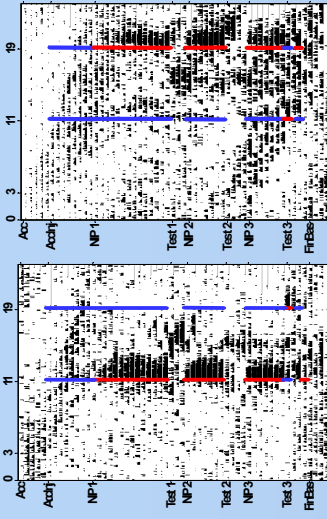
During the nicotine phases, Group Paired received daily saline injections (1 ml/kg) at 11:00, and daily nicotine injections (1 mg/kg) at 19:00. The nicotine injections were paired with a tone (sonalnet) from 19:05 to 19:25. Group Unpaired received daily nicotine injections at 11:00 and saline injections at 19:00 (also with the tone)

**Test 1** - Circadian entrainment effects of injections: No injections and no tones.

**Test 2** - Conditioned effects of paired stimulus: No injections. Tone presented at usual time on Days 1-2, and at a novel time (11:05 - 11:25) on Days 3-4.

**Test 3** - Probe test for effect of injection time: No Tones. Injections at opposite times on Day 1, and at normal times on Day 3. No injections on Days 2 and 4.

## Study Actograms

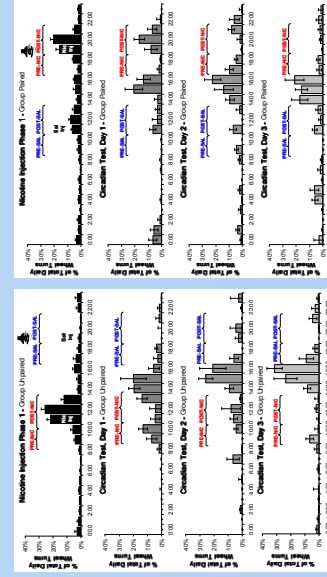


Group Unpaired

Group Paired

Figure 1. Actograms of wheel running activity for both groups. The red lines indicate the nicotine injection times. Saline injection times are indicated with blue lines. The tone was played from 19:05-19:25 during nicotine injection phases, which paired it with the nicotine effects in Group Paired.

## Test 1: Circadian Effects

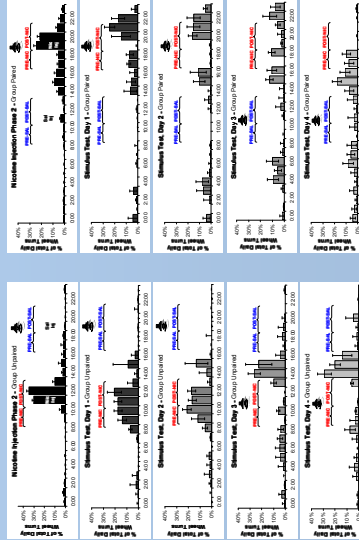


Group Unpaired

Group Paired

Figure 2. Percentage of total daily wheel running for both groups during Nicotine Injection Phase 1 and Test 1. No injections were administered, and the auditory stimulus was not presented during this test phase.

## Test 2: Stimulus Effects

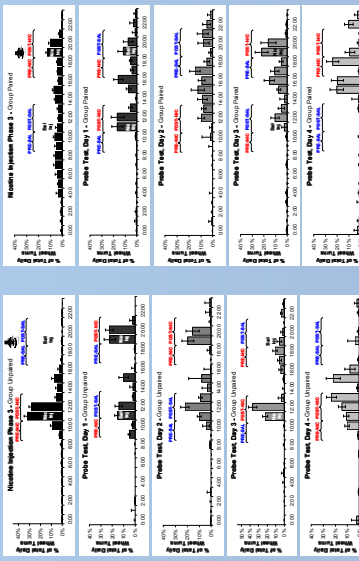


Group Unpaired

Group Paired

Figure 3. Percentage of total daily wheel running for both groups during Nicotine Injection Phase 2 and Test 2. No injections were administered during Test 2. The auditory stimulus was presented at 19:05, the normal presentation time, on Days 1 and 2. On Days 3 and 4, the stimulus was presented at 11:05, a novel time.

## Test 3: Probe Test



Group Unpaired

Group Paired

Figure 4. Percentage of total daily wheel running for both groups during Nicotine Injection Phase 3 and Test 3. The auditory stimulus was not presented during Test 3. Nicotine injections were administered at the normal saline injection time on Day 1, and vice versa. On Day 3, all injections were administered at the normal times. No injections were administered on Days 2 and 4.

## Conclusions:

*Pre-injection (anticipatory) activity* in Group Unpaired, shown in the 2 hours prior to the nicotine injection time, persisted for 2-3 days when injections ceased. In contrast, nicotine anticipatory activity in Group Paired was not significantly greater than activity at other times of day. During Test 3 (with no tone), anticipatory activity in both groups was only observed prior to the nicotine injection time the previous day. The largest amount of anticipatory activity shown in Group Paired was recorded on Test 3, Day 4, following the only day in the study in which this group received a nicotine injection at the normal time without the paired tone.

Both groups showed a *post-injection activity* spike for 2-3 hours after the nicotine injection throughout the study. During Tests 1 and 2 (without injections), both groups continued to show activity at this time on the first two days. Group Paired also showed activity at this time on Test 2, Day 3, when the tone was presented at an earlier, novel time. No response was observed in either group at the novel presentation time. Following novel injection times for nicotine and saline in Test 3, both groups showed activity which persisted the following day. When the injections were administered again at the normal times, little activity was associated with the saline injection, and only the nicotine injection time showed activity on the subsequent day.

These results show that a paired auditory stimulus interferes with circadian anticipatory activity associated with nicotine injections, but does not interfere with the occurrence of the post-injection activity spike. The post-injection activity rhythm can be reset by administering nicotine at a novel time, but not by presenting the stimulus alone at a novel time.

## References:

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