Pharmacology, Biochemistry and Behavior 156 (2017) 10–15

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

# Depression of home cage wheel running is an objective measure of spontaneous morphine withdrawal in rats with and without persistent pain

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ARTICLE INFO

*Keywords:*
Opioid withdrawal
Dependence
Addiction
Tolerance
Locomotor activity

ABSTRACT

Opioid withdrawal in humans is often subtle and almost always spontaneous. In contrast, most preclinical studies precipitate withdrawal by administration of an opioid receptor antagonist such as naloxone. These animal studies rely on measurement of physiological symptoms (e.g., wet dog shakes) in the period immediately following naloxone administration. To more closely model the human condition, we tested the hypothesis that depression of home cage wheel running will provide an objective method to measure the magnitude and duration of spontaneous morphine withdrawal. Rats were allowed access to a running wheel in their home cage for 8 days prior to implantation of two 75 mg morphine or placebo pellets. The pellets were removed 3 or 5 days later to induce spontaneous withdrawal. In normal pain-free rats, removal of the morphine pellets depressed wheel running for 48 h compared to rats that had placebo pellets removed. Morphine withdrawal-induced depression of wheel running was greatly enhanced in rats with persistent inflammatory pain induced by injection of Complete Freund's Adjuvant (CFA) into the hindpaw. Removal of the morphine pellets following 3 days of treatment depressed wheel running in these rats for over 6 days. These data demonstrate that home cage wheel running provides an objective and more clinically relevant method to assess spontaneous morphine withdrawal compared to precipitated withdrawal in laboratory rats. Moreover, the enhanced withdrawal in rats with persistent inflammatory pain suggests that pain patients may be especially susceptible to opioid withdrawal.

## 1. Introduction

The increased use of opioids to treat pain has caused a simultaneous increase in the incidence of opioid dependence (Volkow and McLellan, 2016). Opioid withdrawal symptoms in opioid-dependent pain patients contribute to the continued use and subsequent abuse of opioids (Brodner and Taub, 1978; Fishbain et al., 1992; Hou et al., 2015). Although opioid withdrawal has been well studied in animals, these studies rarely assess spontaneous withdrawal as it occurs in humans (Papaleo and Contarino, 2006; Schulteis, 2010). Administration of an opioid receptor antagonist such as naloxone precipitates immediate and severe physiological withdrawal symptoms (e.g., wet dog shakes, teeth chattering, diarrhea) in opioid-dependent animals (Maldonado et al., 1996), but this phenomenon bears little resemblance to spontaneous opioid withdrawal which appears to induce greater psychological than physiological symptoms and occurs over a prolonged period of time (Schaefer and Michael, 1983; Schulteis et al., 1998; Cicero et al., 2002).

A number of methods have been used to assess spontaneous withdrawal in rodents. These include assessment of physical withdrawal symptoms (Cicero et al., 2002; Kalinichev and Holtzman, 2003; Cobuzzi and Riley, 2011), saccharin consumption (Cobuzzi and Riley, 2011), food intake (van der Laan et al., 1991), anxiety (Schulteis et al., 1998), conditioned place aversion (Vargas-Perez et al., 2009), intracranial self-stimulation (Schaefer and Michael, 1983; Holtz et al., 2015), working memory (Sala et al., 1994), and startle responses (Kalinichev and Holtzman, 2003). Depending on the behavior observed, these studies indicate that spontaneous opioid withdrawal ranges from 3 to 72 h. Unfortunately, capturing the complete time course for withdrawal is not possible with these approaches either because testing is confined to specific times (e.g., physical withdrawal symptoms) or the time of the observed behavior is not evident (e.g., food intake). These tests can also be difficult to use because of prolonged training procedures, invasive surgeries, or the introduction of confounds from removing an animal from its home cage for testing. Given that spontaneous withdrawal signs may occur infrequently and at unpredictable times in the hours or days following cessation of opioid use (Schulteis, 2010), an objective method to assess withdrawal continuously is needed.

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http://dx.doi.org/10.1016/j.pbb.2017.03.007
Received 22 January 2017; Received in revised form 3 March 2017; Accepted 30 March 2017
Available online 31 March 2017

Wheel running is a natural and voluntary rodent behavior. We have previously shown that home cage wheel running provides an objective and clinically relevant measure of the duration and magnitude of pain and opioid analgesia in rats (Kandasamy et al., 2016, 2017a, 2017b). If depression of home cage wheel running is a measure of an abnormal physiological state as these previous studies indicate, then spontaneous opioid withdrawal should also cause depression of home cage wheel running. Although almost all previous preclinical studies of opioid withdrawal examine symptoms in naïve animals, the high incidence of prescription opioid abuse in pain patients (Ballantyne and LaForge, 2007) suggests that pain may exacerbate opioid withdrawal symptoms. These hypotheses will be tested by assessing spontaneous morphine withdrawal in rats with and without persistent inflammatory pain using home cage wheel running.

## 2. Materials and methods

### 2.1. Subjects

Data were collected from 43 adult male Sprague-Dawley rats bred at Washington State University Vancouver. All rats weighed 260–410 g at the start of the study and were randomly assigned to treatment groups. Prior to wheel exposure, rats were housed in pairs in a colony room (22–24 °C) on a reverse 12/12-hour light/dark cycle (lights off at 1700 h). All procedures were approved by the Washington State University Animal Care and Use Committee and conducted in accordance with the International Association for the Study of Pain's Policies on the Use of Animals in Research.

### 2.2. Running wheel

Rats were housed individually to assess wheel running. A Kaytee Run-Around Giant Exercise Wheel (diameter = 27.9 cm; Kaytee Products, Inc., Chilton, WI, USA) was suspended from the top of the rat's home cage. The floor of the cage was covered with cellulose bedding (BioFresh™, Ferndale, WA, USA). A thin aluminum plate (0.8 mm × 5.08 cm × 3.81 cm; K & S Precision Metals, Chicago, IL, USA) was attached to one spoke of the running wheel to interrupt a photobeam projecting across the cage with each rotation. The beam was set 18 cm above the floor of the cage so that only the rotation of the wheel, not the normal activity of the rat, would interrupt the beam. The number of wheel revolutions was summed over 5 min bins for 23 h each day using Photobeam Activity System software (San Diego Instruments, San Diego, CA, USA). Recordings began at the beginning of the dark phase (1700 h) of the day/night cycle when rats are most active. A full description of the running wheel with video is available in our previous publication (Kandasamy et al., 2016). Rats were allowed unrestricted access to the wheel for 23 h/day for 8 days prior to the experimental manipulation. The number of wheel revolutions that occurred during the 23 h during the eighth day was used as the baseline activity. Rats that ran < 400 revolutions on the baseline day were not included in further testing (n = 13/56) (Kandasamy et al., 2016). A diagram of the experimental timeline is provided in Fig. 1.

### 2.3. Pellet implantation surgery

Animals were briefly anesthetized with isoflurane (3–5 min) and implanted with two 75 mg morphine or placebo pellets (NIDA Drug Supply Program, Bethesda, MD, USA). The pellets were wrapped in nylon and implanted subcutaneously in the upper back. The morphine dose was consistent with previous studies examining dependence (Gold et al., 1994). The incision was closed with wound clips and a topical antibiotic was applied. While anesthetized, a subset of animals (Experiments 2 and 3) received an injection of Complete Freund's Adjuvant (CFA, 0.1 mL; Sigma-Aldrich, Inc., St. Louis, MO, USA) into the right hindpaw to induce inflammatory pain. Rats woke up from anesthesia in their home cages where they had continuous access to a running wheel.

### 2.4. Experiment 1: spontaneous withdrawal in naïve rats

The objective of this experiment was to determine whether wheel running could be used to measure tolerance and withdrawal from continuous morphine administration in normal pain-free rats. Wheel running was recorded for three consecutive days following pellet implantation. At the end of the third day, rats were re-anesthetized, the incision was opened, and the nylon covered pellets were removed. The incision was closed with wound clips and the rat was returned to its home cage. Wheel running was assessed 23 h a day for the next 6 days.

### 2.5. Experiment 2: spontaneous withdrawal in inflamed animals after 3 days of morphine

The objective of this experiment was to determine whether chronic inflammatory pain alters the magnitude and/or duration of spontaneous morphine withdrawal as assessed by depression of home cage wheel running. The methodology for this experiment was identical to Experiment 1 except rats received a unilateral injection of CFA into the right hindpaw on the day the pellets were implanted. The effect of implanting and removing two morphine pellets on wheel running was compared to a control group that received two placebo pellets.

### 2.6. Experiment 3: spontaneous withdrawal in inflamed animals after 5 days of morphine

The objective of this experiment was to determine whether prolonged morphine exposure would enhance the magnitude of spontaneous morphine withdrawal in rats with hindpaw inflammation. The methodology for this experiment was identical to Experiment 2 except rats were exposed to morphine or placebo pellets for five instead of three consecutive days.

### 2.7. Data analysis

Baseline activity was defined as the total number of wheel revolutions during the 23 h preceding the first injection. Given individual differences in wheel running, all wheel running data are presented as a percent change from each rat's baseline value. Nearly all running occurred during the dark phase of the daily cycle. The average hourly nighttime running rate was used to calculate the percent change in running when hourly data are reported. All data are expressed as mean ± SEM. Data were analyzed using an independent samples *t*-test (Experiment 1) to compare placebo- and morphine-treated groups or repeated measures ANOVA for day-by-day and hour-by-hour running analyses (Experiments 1–3). Statistical significance was defined as a probability of $< 0.05$.

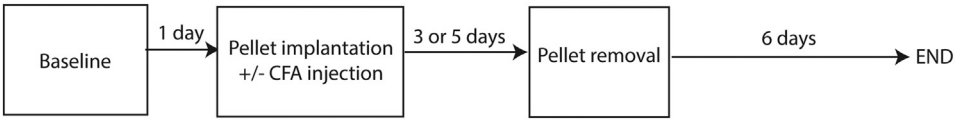


**Fig. 1.** Experimental timeline. Rats in Experiment 1 had pellets removed after 3 days and did not receive CFA injections. Rats in Experiment 2 had pellets removed after 3 days and received CFA injections. Rats in Experiment 3 had pellets removed after 5 days and received CFA injections.