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# A novel method to induce nicotine dependence by intermittent drug delivery using osmotic minipumps

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ABSTRACT

Although osmotic minipumps are a reliable method for inducing nicotine dependence in rodents, continuous nicotine administration does not accurately model the intermittent pattern of nicotine intake in cigarette smokers. Our objectives, therefore, were to investigate whether intermittent nicotine delivery via osmotic minipumps could induce dependence in rats, and to compare the magnitude and duration of withdrawal following forced abstinence from intermittent nicotine to that induced by continuous nicotine administration. In order to administer nicotine intermittently, rats were surgically implanted with saline-filled osmotic minipumps attached to polyethylene tubing that contained hourly unit doses of nicotine alternating with mineral oil to mimic "injections". Three doses of nicotine (1.2, 2.4, and 4.8 mg/kg/day) and saline were administered for 14 days using this method. In order to compare our intermittent delivery method with the more traditional continuous nicotine delivery, a second group of rats was implanted with minipumps attached to tubing that delivered continuous nicotine for 14 days. Rats were administered a 1.5 mg/kg subcutaneous (SC) mecamylamine challenge and observed for somatic signs of withdrawal on days 7, 14, 21, and 28 following minipump implantation. Fifteen somatic withdrawal signs were summed within a 50-minute observation period to obtain a composite Dependence Score. A generalized linear mixed-effects model revealed a significant Day × Dose × Method interaction. Amongst continuously-treated rats, only 4.8 mg/kg/d nicotine resulted in dependence scores significantly greater than those of controls at 14 days of exposure. In contrast, all intermittent nicotine groups showed significantly higher scores beginning at 7 days of exposure and persisting beyond 7 days of abstinence. In general, intermittent delivery produced a more robust withdrawal syndrome than continuous delivery, and did so at a lower dose threshold and with greater persistence after forced abstinence.

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## 1. Introduction

Withdrawal from chronic nicotine usage in humans is generally characterized by irritability/agitation, aggression, anxiety, depression, increase in weight and appetite, restlessness, and craving for nicotine (Hughes et al., 1991). Importantly, these symptoms are often reported to be the proximal cause of relapse to cigarette smoking in those trying to remain abstinent (USDHHS, 1988). A better understanding of the neurobiological changes that occur as a consequence of nicotine dependence and withdrawal may aid in the development of better preventative and treatment intervention therapies.

Appropriate preclinical models of drug dependence allow for in-depth, mechanistic examination of susceptibility to and factors responsible for addiction, and for developing potential treatment therapies prior to human testing. Self-administration (Paterson and Markou, 2004), daily intraperitoneal (IP) or subcutaneous (SC) nicotine injections (Clarke and Kumar, 1983; Pandey et al., 2001), oral nicotine administration via drinking water (Flynn et al., 1989), and osmotic minipumps (Besheer and Bevins, 2003; Malin et al., 1992) have commonly been employed to induce nicotine dependence in rodent models. The most frequently employed nicotine administration method is the osmotic minipump, which is implanted SC or IP and infuses nicotine continuously. Osmotic minipumps overcome many of the challenges associated with other methods, including lengthy training, costly equipment, or stress and conditioned drug effects induced by repeated drug injections.

While the minipump method obviates many of the limitations inherent to those described above, it too has a notable limitation — its continuous mode of drug delivery. One of the main drawbacks of using continuous nicotine infusion to induce dependence is that this delivery method does not closely mimic the intermittent pattern of nicotine intake typical of human smokers—i.e., smoking a cigarette for a few minutes every few hours throughout the day, with an extended period of withdrawal during sleep. This intermittent pattern of human nicotine administration is thought to allow nicotinic acetylcholine receptors (nAChRs), which undergo rapid upregulation but desensitization, to

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return to their full active state during the administration period (Marks et al., 1993). This is arguably a very important aspect of nicotine tolerance and withdrawal development and maintenance (Dani, 2001).

Because the aversiveness of nicotine withdrawal plays an important role in the maintenance of its use amongst human smokers, rodent models of nicotine withdrawal have become a widely-used means of assessing the degree of dependence amongst nicotine-treated animals. Malin et al. (1994) first demonstrated that an acute injection of the nAChR antagonist mecamylamine induces a nicotine withdrawal syndrome in rats receiving chronic nicotine via osmotic minipump, and others (e.g., Hildebrand et al., 1997) have confirmed this finding. The severity of withdrawal symptoms has also been found to correspond to the amount of prior nicotine exposure (Malin et al., 1992). To our knowledge, no study has yet examined the mecamylamine-precipitated withdrawal syndrome in rats receiving passive, intermittent nicotine for an extended period of time.

As such, the goal of the present study was to adapt and validate a method for inducing nicotine dependence in rats via automatic, repeated, intermittent injections of nicotine over a duration of 14 days using osmotic minipumps. Additionally, we sought to compare the dependence induced by this novel method to dependence induced by continuous administration via the same device. Nicotine dependence was assessed behaviorally, and quantified by summation of somatic behavioral signs of withdrawal following an acute challenge of mecamylamine.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats (Charles River Laboratory) weighing 300–325 g were single-housed under a 12-h light/dark cycle (0600 h lights on). N = 13–15 rats per group were administered intermittent nicotine or saline, and n = 6 rats per group were administered continuous nicotine. Animals had ad libitum access to food and water and were handled for 1 week prior to experimentation. The Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program approved all procedures used in the study.

### 2.2. Drugs

(−)-Nicotine hydrogen tartrate and mecamylamine hydrochloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Nicotine was dissolved in 0.9% sodium chloride (Hospira) to achieve the desired concentration, and pH was adjusted to 7.2 (±0.5) with sodium hydroxide (Sigma Aldrich).

### 2.3. Nicotine administration

We developed an automated method to non-continuously deliver discrete nicotine "injections" for 14 days using an osmotic minipump. Our goal was to deliver a single injection of nicotine every other hour, 24 h/day for 2 weeks. We accomplished this by forming a long piece of PE-60 tubing (Instech Laboratories, Plymouth Meeting, PA, USA) into a coil using a method first described by Lynch et al. (1980) and prefilling the coil with nicotine solutions separated by mineral oil (Fig. 1A). Taking into account the inner diameter of the PE-60 tubing and the 2.5 μl/h flow rate of the osmotic minipump (model 2ML4; Alzet, Palo Alto, CA, USA), we calculated the total length of tubing needed for 14 days of infusions (187 cm) and the drug concentrations needed for each infusion period given the constant injection volume.

Once the coils were formed, they were filled with nicotine (2.5 μl) and mineral oil (2.5 μl) in an alternating fashion using a Y-connector (Instech Laboratories) and two infusion pumps (Harvard Apparatus) to deliver precise volumes. In a second cohort of rats, coils were filled with only nicotine solution to allow for comparison of intermittent delivery with continuous delivery. The osmotic minipump, which contains a reservoir that holds enough solution for 28 days of delivery, was filled with saline and primed for 24 h in 37 °C saline for both method groups.

Prior to implantation for IP drug administration, the prefilled coil was attached to the flow moderator of the minipump (Fig. 1B). Because it was determined in vitro that the length of the filled coil produced backpressure sufficient to cause leakage of saline from the pump at the point of connection to the coil, Instant Krazy Glue (Elmer's Products Inc., Columbus, OH, USA) was applied below the cap of the flow moderator to seal it to the top of the pump and to the end of the coil once it had been attached to the flow moderator. Additionally, a 1.5 cm-long piece of 1.6 mm diameter heat-shrink tubing (RadioShack Corp., Fort Worth, TX, USA) was placed at the end of the coil where it was attached to the flow moderator in order to prevent the coil from cracking when animal movement created additional stress at the point of connection. A soldering station (Weller model WSD81, Germany) heated to 700 °F was used to seal the heat-shrink tubing around the coil. The length of the coil was then wrapped around the minipump (Fig. 1C), and the device was implanted immediately. A pilot in vitro study confirmed that the flow rate of nicotine and oil through the coil was the same as the expected flow rate of solution from a minipump used without a catheter (2.5 μl/h).

In order to compare the intermittent delivery method with continuous infusion of nicotine, we implanted an additional group of rats (n = 6/group) with saline-filled minipumps attached to Lynch coils that were only filled with nicotine solution. These group sizes, though smaller than the intermittent groups, are comparable in size to those of other nicotine behavioral studies (e.g. Malin et al., 1994). We selected three nicotine doses from the literature reflecting a high (0.4 mg/kg every other hour or 0.2 mg/kg/h continuously for a total of 4.8 mg/kg/day), moderate (0.2 mg/kg every other hour or 0.1 mg/kg/h continuously for a total of 2.4 mg/kg/day; Benwell et al., 1995) and low (0.1 mg/kg/h every other hour or 0.05 mg/kg/h continuously for a total of 1.2 mg/kg/day; Malin et al., 1992) dose previously shown to induce dependence; doses are expressed as the free-base. Control animals were implanted with minipumps attached to coils filled with alternating saline and oil. Following the last day of testing, rats were sacrificed and the integrity of the tubing and pump was assessed.

### 2.4. Osmotic minipump implantation

Rats were anesthetized with 2–3% isoflurane in a 1 to 1 mixture of $O_2$ and air. An incision was made in the abdomen large enough to fit the pump and tubing inside the peritoneal cavity. Gut sutures were used to close the muscular peritoneal layer and wound clips were used to close the skin incision. Surgical procedures were performed according to an aseptic protocol.

### 2.5. Somatic signs of nicotine withdrawal

An acute injection of mecamylamine HCl (1.5 mg/kg, SC) was administered at 7, 14, 21, and 28 days after pump implantation to precipitate nicotine withdrawal (note: nicotine was delivered only for the first 14 days of the 28 days of minipump capacity). Rats were observed for withdrawal signs in square Plexiglas chambers (35 × 35 × 40 cm). They were habituated to the chambers for 10 min/day 3 days prior to testing. On the day of testing, an additional 10-min habituation period was followed by a 60-min observation period: 10-min baseline before and 50 min precipitated withdrawal immediately following mecamylamine administration. The following somatic signs were tallied within 10-min intervals during the test (Malin et al., 1992): teeth chattering, chewing, gasping, writhing, head shakes, body shakes, tremors, blinks, yawns, seminal ejaculation, genital licks, hind foot scratches, escape attempts, and ptosis. Ptosis, if present, was counted only once per minute. Withdrawal behaviors were scored by two raters, who were blinded to the drug condition. There was over 90% inter-rater reliability of the behavioral scoring between observers.