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# L-DOPA attenuates nicotine withdrawal-induced behaviors in rats

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ABSTRACT

There is some evidence that during nicotine abstinence brain dopamine levels are reduced. The hypothesis for the present study was that the precursor amino acid L-DOPA would relieve nicotine withdrawal-induced behaviors. Separate groups of adult male Sprague–Dawley rats were used. (−)-Nicotine bitartrate (9 mg/kg/day, salt content) or equimolar sod ium tartrate was infused into each rat via a subcutaneous osmotic minipump for 7 days. To assess nicotine withdrawal behaviors, locomotor activity was measured for 24 h in their home cage. Somatic signs were also counted approximately 22 h after pump removal. Moreover, depressive-like behaviors were evaluated in the forced swimming test approximately 48 h after pump removal. One day after removal of pumps, locomotor activity was suppressed in nicotine-infused rats compared to the tartrate-infused controls. Somatic signs of nicotine withdrawal were increased in nicotine-infused rats compared to the controls. Two days after removal of pumps, increased immobility in the forced swimming test was observed in abstinent nicotine-infused rats as compared with controls. The administration of L-DOPA methyl ester (equivalent to 50 mg/kg L-DOPA, s.c.) and benserazide (10 mg/kg, s.c.) attenuated somatic signs of withdrawal and reversed nicotine withdrawal-induced depressive-like behaviors in the forced swimming test. It did not mitigate nicotine withdrawal-induced locomotor suppression in the animals' home cages. These results indicate that L-DOPA could be a useful agent to alleviate some nicotine withdrawal symptoms.



## 1. Background

Abrupt tobacco cessation in chronic smokers causes withdrawal symptoms including anxiety, craving for tobacco, difficulty in concentrating, depressed mood, increased appetite, insomnia, fatigue, decreased arousal, and irritability (Hughes, 2007, 2008; Malin and Goyarzu, 2009; American Psychiatric Association, 2000; World Heatlh Organization, 1993). Alleviating these symptoms may help smokers quit smoking. Depressed mood and craving for tobacco predict relapse while other symptoms are relatively weak predictors of relapse (review, Hughes, 2007). However, irritability is the most frequently reported symptom(s) during smoking cessation (Hughes, 2007).

Abrupt withdrawal from repeated nicotine decreases basal levels of dopamine (DA) release in rat nucleus accumbens (Takahashi et al., 1998; Rahman et al., 2004). Mecamylamine precipitates nicotine withdrawal and significantly decreases extracellular DA in nucleus accumbens though it is not clear whether the decreased DA release reflects decreased basal DA levels or attenuated nicotine-induced DA release (Hildebrand et al., 1998; Rada et al., 2001). Domino and Tsukada (2009) also found that basal levels of DA release in the monkey dorsal striatum were decreased after overnight abstinence from daily nicotine. However, after overnight abstinence, nicotine induced DA release was enhanced over controls, indicating sensitization and not attenuation. In humans, smokers abstinent from tobacco for 11 to 17 h have only 54% of the cerebrospinal fluid concentration of the DA metabolic homovanillic acid (HVA) as compared with nonsmokers (Geracioti et al., 1999).

Based on the above findings, we examined whether L-DOPA, a precursor of DA, is effective for relieving nicotine withdrawal-induced behaviors. If decreased DA levels after nicotine abstinence were a cause of nicotine withdrawal, a precursor of DA should be effective for relieving withdrawal signs. As a measure of nicotine withdrawal, we used nicotine withdrawal-induced rat locomotor depression (Gäddnäs et al., 2000; Catania et al., 2003) and somatic signs of withdrawal (Malin et al., 1992). Somatic signs of nicotine withdrawal are considered to reflect mainly irritability (Malin and Goyarzu, 2009). The rat forced swimming test was also employed as an animal model suggested to predict or evaluate mood changes in emotional state (Porsolt et al., 1977, 1978). The acute nicotine abstinence syndrome in rats does not exactly duplicate tobacco smoking withdrawal in humans. Nevertheless, it does provide quantitative measures that do replicate some of the predominant mood changes of smoker tobacco abstinence and how much they are modified by L-DOPA in an animal model.

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## 2. Methods

### 2.1. Animals

Male Sprague Dawley rats (Harlan, Indianapolis, IN), weighing 260–320 g in the beginning of the experiment were housed 2–3 per cage at a constant temperature of 20–21 °C. Animals were maintained on a 12 h light:dark cycle (lights on at 7:00, lights off at 19:00). Each animal had free access to rodent chow and water. Animal treatment complied with the NIH Animal Care Guidelines, and all procedures were performed according to a protocol approved by the University of Michigan Committee for Use and Care of Animals. Fig. 1 delineates the timeline that was used for the overall experimental design across days as described below.

### 2.2. Drugs

(−)-Nicotine bitartrate salt, sodium tartrate, benserazide, and L-DOPA methyl ester were purchased from Sigma-Aldrich, St. Louis, MO, USA. Each compound was dissolved in saline. The pH of the solution was adjusted using NaOH to approximately 7.0. Equimolar sodium tartrate dissolved in saline was used as the control solution for nicotine solution. All drugs for acute injection were administered s.c. in a volume of 1 ml/kg.

### 2.3. Surgical procedure

#### 2.3.1. Osmotic minipump implantations and removals

For chronic nicotine administration, osmotic minipumps (Model 2ML2, Durect Corporation, Cupertino, CA, USA) for drug infusion surgically were implanted s.c. between the scapulae under ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. The pumps were filled with either control solution or nicotine. The nicotine concentration was adjusted to deliver a dose of 9 mg/kg/day of nicotine salt (3.16 mg/kg/day nicotine base). Nicotine or the control solution was infused via implanted pumps at 5 μl/h for 1 week. This dose and duration has been used in previous studies of nicotine withdrawal (Malin et al., 1992; Hamilton et al., 2009, 2010). Moreover, the blood concentrations resulting from this dose in rats are almost same concentrations as those measured in heavy smokers (Benowitz et al., 1982; LeSage et al., 2002). One week after implantation of osmotic minipumps, minipumps were surgically removed under ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia.

#### 2.3.2. Telemetry device implantations for locomotor activity

Telemetry devices (model ER-4000 E-Mitter; Mini Mitter Co., Bend, OR) were placed into the abdomen at the same time as osmotic pumps implant. The radiotransmitters were implanted inside the peritoneal cavity sending radio signals to a receiver (Model ER-4000 Receiver; Mini Mitter Co.) placed under the home cage of each rat. Data were collected and processed simultaneously by the Vital View data acquisition system (Mini Mitter Co.). This method was used previously to measure the effects of drugs on locomotor activity (e.g., Chen et al., 2007; Jutkiewicz et al., 2008).

### 2.4. Spontaneous somatic signs of nicotine withdrawal

Behavioral observations were performed from 9 to 12 am in a clear plastic observation chamber (48 × 23 × 20 cm). We observed behaviors 20–24 h after pump removal, based on previous studies (Malin et al., 1992; Rylkova et al., 2008). Rats' behaviors were counted for 30 min by observers who were blind to the experimental condition. Teeth-chattering/chews, stretches/gasps, shakes, ptosis, and miscellaneous other less frequent signs (e.g. diarrhea and yawns) were counted.

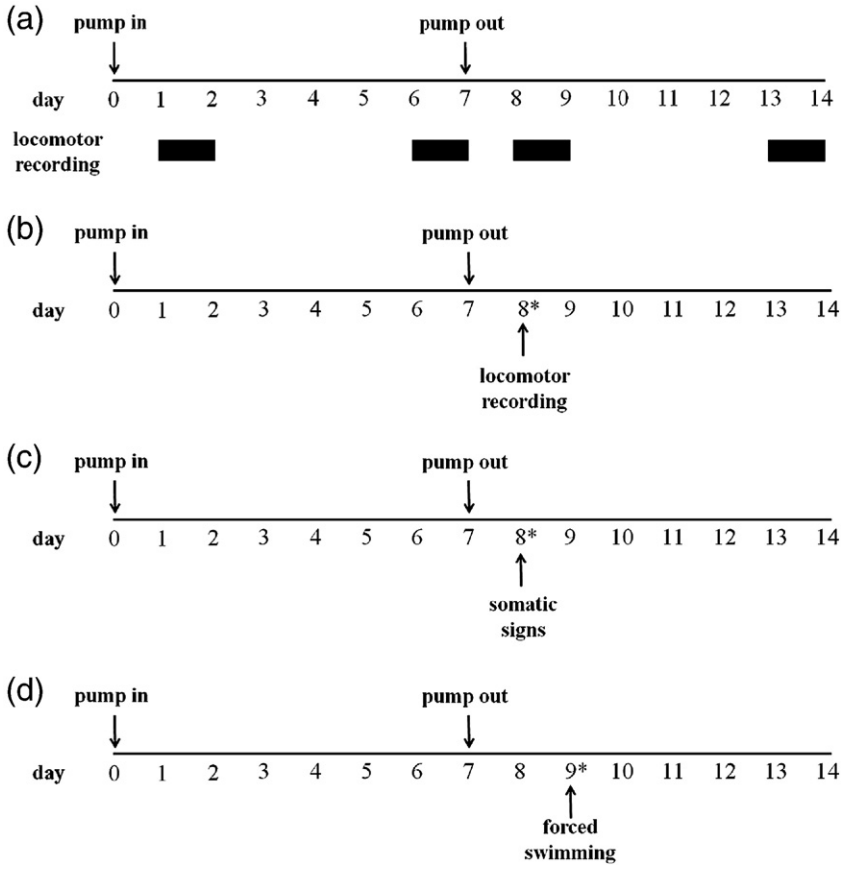


**Fig. 1.** Timeline of experimental designs for experiments 1 (a), 2 (b), 3 (c) and 4 (d).