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Research report

# Sex differences in attenuation of nicotine reinstatement after individual and combined treatments of progesterone and varenicline

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## HIGHLIGHTS

- Male and female rats had no differences during maintenance and extinction.
- Both males and females showed reinstatement to the CAF + CUES condition.
- Male rats, but not females, reinstated to the NIC + CUES condition.
- Varenicline alone attenuated NIC + CUES reinstatement in males.
- Combination varenicline and progesterone decreased NIC + CUES reinstatement in males.

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## ABSTRACT

Tobacco use is the largest cause of preventable mortality in the western world. Even after treatment, relapse rates for tobacco are high, and more effective pharmacological treatments are needed. Progesterone (PRO), a female hormone used in contraceptives, reduces stimulant use but its effects on tobacco addiction are unknown. Varenicline (VAR) is a commonly used medication that reduces tobacco use. The present study examined sex differences in the individual vs. combined effects of PRO and VAR on reinstatement of nicotine-seeking behavior in a rat model of relapse. Adult female and male Wistar rats self-administered nicotine (NIC, 0.03 mg/kg/infusion) for 14 days followed by 21 days of extinction when no cues or drug were present. Rats were then divided into 4 treatment groups: control (VEH + SAL), PRO alone (PRO + SAL), VAR alone (VEH + VAR) and the combination (PRO + VAR). Reinstatement of nicotine-seeking behavior induced by priming injections of NIC or caffeine (CAF), presentation of cues (CUES), and the combination of drugs and cues (e.g. NIC + CUES, CAF + CUES) were tested after extinction. Male and female rats did not differ in self-administration of nicotine or extinction responding, and both showed elevated levels of responding to the CAF + CUES condition. However, males, but not females, reinstated active lever-pressing to the NIC + CUES condition, and that was attenuated by both VAR and VAR + PRO treatment. Thus, males were more sensitive to NIC + CUE-induced reinstatement than females, and VAR alone and VAR combined with PRO effectively reduced nicotine relapse.



## 1. Introduction

Tobacco use is the single largest preventable cause of mortality in the western world [1], and it is characterized by abstinence periods followed by relapse [2]. A significant challenge in treating tobacco addiction is preventing relapse as only about 5% of smokers remain abstinent without treatment [2,3]. With treatment, abstinence rates still remain low with only 10–30% of smokers remaining tobacco-free long-term [4]. Since permanent tobacco cessation is necessary to limit the negative health consequences of tobacco use, more effective treatments to reduce relapse and maintain long-term cessation are necessary.

Progesterone (PRO) has shown promise in reducing smoking-related behavior. When endogenous PRO levels were high, motivation for nicotine (NIC), the primary psychoactive component of tobacco, was decreased in humans [5], nonhuman primates [6] and rats [7]. Conversely, in studies where endogenous PRO levels were low, rats showed greater motivation for NIC [8]. Increased plasma levels of PRO were associated with a 23% increase in the incidence of abstinence for each additional week of treatment in humans [9]. Exogenously-administered PRO also decreased

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self-administration and reinstatement (e.g. relapse) to cocaine, another commonly abused stimulant [10–14]. However, the effects of exogenous PRO on relapse to NIC use have not yet been examined.

Varenicline (VAR) has also shown efficacy for treatment of tobacco use. Varenicline is an α4β2 nicotinic receptor partial agonist and α7 nicotinic receptor full agonist that is currently approved by the Food and Drug Administration for smoking cessation [15]. Varenicline has little to no abuse liability [16], and it is considered a safe and effective treatment for tobacco addiction [17]. Varenicline doubled to tripled successful tobacco cessation attempts compared to no treatment [18]. In rats, VAR also decreased the reinforcing effects of NIC, shown by attenuation of NIC self-administration and lower breakpoints for NIC on a progressive-ratio (PR) schedule [19,20]. Varenicline also blocked reinstatement of drug-seeking induced by NIC [21] and cues previously associated with NIC [19,22], supporting clinical findings of VAR's ability to reduce relapse to smoking.

Recent studies have shown that combination therapies may be a more effective method for increasing abstinence rates than individual treatments [23,24]. Combination therapies were associated with higher levels of abstinence compared with monotherapies [25–29], even with treatments from different pharmacological classes such as varenicline and bupropion [30,4]. Importantly, combination therapies did not increase risk to patients, as adverse reaction rates were similar between the individual and combined therapies [23]. Thus, combined treatments are a promising and safe method for reducing relapse to NIC use. Since both PRO and VAR was effective when used as individual treatments, the goal of the present research was to combine therapies in a nicotine relapse model in rats. Previous work had shown that the combination of PRO and another treatment, atomoxetine, was more beneficial than the individual treatments in reducing cocaine-seeking [31]. Similarly, the combination of a behavioral intervention (e.g. wheel-running) and PRO showed additive effects [32]. The combination of VAR with treatments such as NIC replacement [23] and bupropion [30,33] in humans has also been more successful than either treatment alone, similar to preclinical findings [34].

Finally, since males and females show unique patterns of relapse to tobacco addiction [35] as well as differential sensitivity to treatments for nicotine addiction, a goal of the present study was to compare effects of single and combined treatments in male and female rats. With other stimulants such as cocaine, females showed enhanced responsivity to reinstatement of cocaine-seeking compared to males [11,36–38]. However, there are limited preclinical data on sex differences in nicotine reinstatement. In the one study on the topic, no sex differences in reinstatement were found to nicotine, cues, a pharmacological stressor or the combination of drugs + cues [39]. Finally, both VAR and PRO have shown sex-specific treatment effects for attenuating NIC and other forms of stimulant use [11,32,41,42], with females showing better treatment effects than males. Thus, sex differences on overall levels of reinstatement, as well as potential sex-specific effects on the interaction between treatment and nicotine-seeking, need to be considered.

Not only is there limited research on sex differences in reinstatement of NIC-seeking to NIC with or without the addition of cues, sex differences in reinstatement to other drugs such as caffeine (CAF) have not been explored. Caffeine (CAF) produced robust reinstatement to NIC-seeking, and, similar to NIC, this effect was enhanced by the addition of CUES [40]. Reinstatement to CAF and CAF + CUES has only been tested in males; thus, it is not known whether there are sex-specific effects of CAF on reinstatement. In our recent work, a CAF priming injection enhanced reinstatement to cocaine-seeking behavior in females but not males [65]; however, it is unknown whether this enhanced sensitivity to CAF would transfer to NIC-seeking as well. Thus, one objective of the present research was to test sex differences in reinstatement of nicotine-seeking behavior to a CAF priming condition.

The overall goals of the present study were twofold: 1) determine sex differences in an animal model of nicotine relapse (e.g. reinstatement) and 2) compare males and females on individual and combined effects of PRO and VAR on nicotine-seeking during reinstatement generated by NIC, CAF, CUES, and their combinations. Females showed greater reinstatement to drug and cue-induced reinstatement to other stimulants [e.g. cocaine;11,36,37,38] than males; thus, it was hypothesized that females would be more sensitive than males to all types of reinstatement (e.g. CUES, NIC, CAF, NIC + CUES, and CAF + CUES). Females also show enhanced treatment effects for stimulant use [11,32,41,42]; thus greater effects of both individual and combined treatments were hypothesized to occur in females compared with males.

## 2. Methods

### 2.1. Animals

Forty-seven female and 48 male drug-naïve Wistar rats (weighing 200–224 g for females and 250–274 g for males on arrival) were used as subjects. The age of the rats was between 63 and 77 days upon arrival from Harlan Sprague-Dawley Inc. (Madison, WI), with females and males matched for age. Acquisition and maintenance data from a subset of rats (approximately half of the males and females) were analyzed for acquisition differences and published as part of a separate study [43]. Upon arrival, all rats were pair-housed in plastic cages and habituated to the laboratory for at least 3 days with *ad libitum* access to food (Teklad 2018, Harlan Laboratories, Madison, WI) and water prior to beginning experiments. Food was then restricted to 20 g (male) and 16 g (female) that had been shown to maintain rats at 85% free-feeding weight, and water was available *ad libitum*. Animal health was checked daily, and rats were weighed weekly. Rats were tested in operant conditioning chambers at the same time each day in batches from 0900 to 1300 h, with food given at 1515 h. Daily sessions took place during the light phase of the light/dark cycle (lights on from 0600 to 1800 h), with rooms maintained at 40–60% humidity and temperatures ranging from 22–23 °C. All experiments were approved by the Institutional Animal Care and Use Committee (Protocol #1307-30762A) and were conducted in accordance with Principles of Laboratory Animal Care [44].

### 2.2. Apparatus

At the beginning of the experiments, rats were individually housed in hanging stainless steel cages and transferred to the same octagonal operant conditioning chamber for each daily session. This procedure was previously described by Anker et al. [45]. Experimental chambers were housed in sound-attenuating custom-built enclosures with a ventilation fan to provide background noise and airflow. Each individual chamber contained two levers located on opposing sides of the chamber with a stimulus light (4.76 W) above each lever. A house light (4.76 W) was located in the upper corner of the chamber. A syringe pump (pH M-100, Med Associates, St. Albans, VT) was attached to a swivel tether system (375/22PS, Instech, Plymouth Meeting, PA; C313CS-MN, Plastics One, Roanoke, VA) to deliver NIC infusions. The tether attached to the rat using a spring-covered harness (CIH95AB, Instech). Data were collected and stored on PCs running MED-PC IV (v. 4.3) software.