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Short communication

Withdrawal from chronic nicotine and subsequent sensitivity to nicotine challenge on contextual learning

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HIGHLIGHTS

- Nicotine withdrawal impaired learning.
- Nicotine challenge enhanced learning.
- The greatest sensitivity to nicotine challenge was observed in nicotine-withdrawn mice.

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ABSTRACT

Nicotine withdrawal is associated with numerous symptoms including impaired hippocampus-dependent learning. Theories of nicotine withdrawal suggest that nicotinic acetylcholine receptors (nAChRs) are hypersensitive during withdrawal, which suggests enhanced sensitivity to nicotine challenge. Research indicates that prior exposure to nicotine enhances sensitivity to nicotine challenge, but it is unclear if this is due to prior nicotine exposure or specific to nicotine withdrawal. Therefore, the present experiments examined if prior nicotine exposure or nicotine withdrawal altered the effects of nicotine challenge on hippocampus-dependent learning. C57BL/6J mice were trained and tested in contextual conditioning following saline or nicotine challenge either during (24 h after cessation) or after (14 days after cessation) a period of nicotine withdrawal symptoms. Nicotine challenge produced a greater enhancement of contextual conditioning relative to control withdrawal state in mice withdrawn from chronic nicotine for 24 h compared to 14 days and corresponding saline controls. These experiments support the suggestion that during periods of abstinence, smokers may perceive tobacco providing a large boost in cognition.

In the United States, cigarette smoking results in 443,000 premature deaths per year despite known negative health consequences [1]. For many smokers, avoidance or alleviation of aversive withdrawal symptoms may contribute to continued nicotine use and relapse during abstinence [2]. One such withdrawal symptom is impaired cognitive function, which is reported to occur in a majority of smokers [3]. In fact, working memory deficits during abstinence predicted smoking resumption [4]. Therefore, understanding the neurobiology of nicotine withdrawal-related changes in cognition will aid in developing better therapeutics for maintaining abstinence.

A great deal of information concerning nicotine's effects on cognition has come from mouse models [5]. In mice, nicotine withdrawal impaired hippocampus-dependent, but not hippocampus-independent, learning while acute nicotine had an enhancing effect [6,7]. Both the enhancing effect of acute nicotine and the impairing effect of nicotine withdrawal on learning are mediated by hippocampal β2-containing nAChRs, likely the α4β2* nAChR (* indicates other subunits may be incorporated) [8,9]. The withdrawal-related impairment in hippocampus-dependent learning is hypothesized to be a result of chronic nicotine upregulating hippocampal α4β2* nAChRs [10]. Chronic nicotine upregulates α4β2* nAChRs in the hippocampus, and this upregulation persists for extended periods of time after cessation of nicotine exposure [10,11]. In addition to upregulation, chronic nicotine also desensitizes nAChRs [12]. During periods of withdrawal, desensitized nAChRs may regain function while remaining upregulated. The recovery of nAChR function during withdrawal coupled with continued upregulation may contribute to a hypersensitive nAChR system. Indeed, as previously hypothesized [13], because of the increased number of nAChRs that are

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responsive during withdrawal, some cholinergic systems may become hyperexcitable, which could alter normal function of the hippocampus leading to impaired learning [10].

A hypersensitive nAChR system may produce enhanced sensitivity to nicotine during withdrawal. In support, repeated nicotine exposure produces sensitized locomotor responses [14,15] and increased neurotransmitter release [15,16] in response to challenge doses of nicotine. However, these studies utilized repeated injection schedules, which may result in periods of activation, desensitization, and resensitization of nAChRs rather than chronic, continuous nicotine exposure that results in a near complete saturation and desensitization of nAChRs. As nAChR desensitization is suggested to be critically involved in nicotine withdrawal [17], it is unclear if the sensitized responses to nicotine were due to prior nicotine exposure or nicotine withdrawal [14–16]. In addition, none of these studies examined the effects of nicotine on hippocampus-dependent learning. To this end, the present experiments examined if nicotine withdrawal or prior nicotine exposure produces enhanced sensitivity to the effects of nicotine challenge on hippocampus-dependent learning.

Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) 8–12 weeks at pump implantation were housed 1–4 per cage with *ad libitum* access to food and water. A 12-h light/dark cycle was maintained from 7:00 AM to 7:00 PM with all experiments conducted during the light cycle. The Temple University Institutional Animal Care and Use Committee approved all experimental procedures.

Mice were implanted with minipumps (Alzet, Model 1002, Durect Co, Cupertino, CA) that delivered chronic saline or nicotine (6.3 mg/kg/d, freebase weight, s.c.) for 12 days. This dose and route of administration was chosen because it produces plasma nicotine levels in the range observed in smokers [6]. In addition, because of the fast half-life of nicotine in the mouse, minipumps produce similar steady state receptor occupancy as in smokers [18]. In all experiments, minipumps were removed 12 days after implantation to induce spontaneous withdrawal. Surgery was performed under sterile conditions with 5% isoflurane anesthetic.

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) dissolved in 0.9% saline was administered i.p. 2–4 min prior to training and testing of contextual conditioning. Doses were saline, 0.022, 0.045, 0.09, 0.18, or 0.36 mg/kg nicotine (freebase weight, based on previous work [7]).

Training and testing of contextual conditioning was performed in four identical clear Plexiglas chambers housed in sound attenuating boxes; as described elsewhere [19]. Freezing, defined as the absence of all movement except respiration, was sampled for 1 s every 10 s and served as a measure of learning. During training, mice were placed in the chambers and baseline freezing was recorded during the first 120 s of the session. At 148 s, mice were presented with a 2 s, 0.57 mA footshock. At 298 s, an additional 2 s footshock was presented. The mice remained in the chambers for 30 s after the second shock presentation. Approximately 24 h later, testing of contextual conditioning occurred in the training context in the absence of the footshock and freezing was scored for 5 min.

Two experiments were performed that are depicted in Fig. 1. Experiment 1 (Fig. 1A) examined the effects of saline or nicotine challenge on learning in mice undergoing nicotine withdrawal symptoms. On day 12 of treatment, minipumps were removed from all mice to induce spontaneous withdrawal. Approximately 24 h later (day 13), mice were randomly assigned to saline or nicotine challenge groups and then were trained in contextual conditioning following injections of saline (withdrawal from chronic saline [WCS] $n = 15$; withdrawal from chronic nicotine [WCN] $n = 14$), 0.022 mg/kg (WCS $n = 13$; WCN $n = 13$), 0.045 mg/kg (WCS $n = 13$; WCN $n = 13$), 0.09 mg/kg (WCS $n = 13$; WCN $n = 16$), 0.18 mg/kg (WCS $n = 14$; WCN $n = 13$), or 0.36 mg/kg (WCS $n = 11$; WCN $n = 10$) nicotine challenge. On day 14, mice were injected again with their respective treatment and tested for contextual conditioning. Training day was chosen as the reference day because previous work showed that 24 h of withdrawal impaired contextual learning [6], but not recall [20]. Experiment 2 (Fig. 1B) examined the effects of saline or nicotine challenge on learning in mice previously exposed to chronic nicotine treatment. On day 12, minipumps were removed from all mice. On day 26, mice were randomly assigned to saline or nicotine challenge groups and then were trained in contextual conditioning following injections of saline (WCS $n = 14$; WCN $n = 14$), 0.022 mg/kg (WCS $n = 13$; WCN $n = 12$). 0.045 mg/kg (WCS $n = 16$; WCN $n = 13$), 0.09 mg/kg (WCS $n = 12$; WCN $n = 15$), 0.18 mg/kg (WCS $n = 12$; WCN $n = 13$) or 0.36 mg/kg (WCS $n = 11$; WCN $n = 13$) nicotine challenge. On day 27, mice were injected again with their respective treatments and tested for contextual conditioning. The 14-day time point was chosen because withdrawal-related changes in contextual conditioning were no longer present at that point [10].

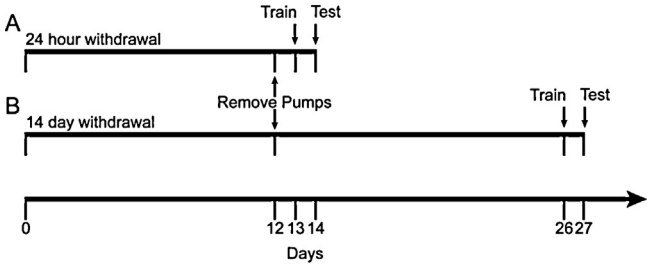

Fig. 1. Timeline of the experimental procedures. (A) Experiment 1: minipumps were implanted on day 0 and removed on day 12. Training and testing occurred on days 13 and 14, respectively. (B) Experiment 2: minipumps were implanted on day 0 and removed on day 12. Training and testing occurred on days 26 and 27, respectively.

To compare contextual freezing from testing day across each experiment, freezing data were converted to percent change from control then analyzed using a three-way ANOVA (time point [24 h, 14 days] × withdrawal [WCS, WCN] × nicotine challenge dose [0.022, 0.045, 0.09, 0.18, 0.36 mg/kg]). Percent change from control was calculated and defined as: percent change from control = $[(F_i - F_c)/(F_c)] \times 100$, where F_i = mean freezing of individual nicotine challenge doses (0.022–0.36 mg/kg) and F_c = mean group freezing of respective controls (either WCS or WCN with saline challenge within each time point). A significant ANOVA was followed by Bonferroni corrected planned contrasts and Tukey's or Games-Howell post hoc tests. Control mice in each condition exhibited no baseline freezing on training day, therefore percent change from control for baseline freezing could not be calculated. Thus, data were reverted into raw values to analyze baseline freezing as well as the withdrawal × challenge interaction from testing day within each time point with two-way ANOVAs. Significant ANOVAs were followed by Tukey's or Games-Howell post hoc tests. Animals with 2.5 standard deviations from the mean were excluded as outliers (9 animals).

A three-way ANOVA on percent change from control revealed a significant time point × withdrawal × nicotine challenge interaction, $F(4, 239) = 2.680$, $p = 0.032$ (Fig. 2). Bonferroni corrected planned contrasts revealed that 24 h WCS mice had a smaller percent change from control than 14 day WCS mice ($p < 0.001$). This trend was reversed in WCN mice whereby 24 h WCN mice had a greater percent change from control than 14 day WCN mice ($p < 0.001$). Overall, 24 h WCN mice showed a greater nicotine challenge-induced percent change from control than all other withdrawal groups ($p < 0.001$). Bonferroni corrected planned contrasts also revealed a significant time point × withdrawal interaction within 0.18 and 0.36 mg/kg nicotine challenge ($ps < 0.05$). Post hoc tests revealed that 24 h WCN mice that received 0.18 or 0.36 mg/kg