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Voluntary co-consumption of alcohol and nicotine: Effects of abstinence, intermittency, and withdrawal in mice

Kyu Y. O'Rourke, Jillienne C. Touchette, Elizabeth C. Hartell, Elizabeth J. Bade, Anna M. Lee*

Department of Pharmacology, University of Minnesota, Minneapolis, MN, 55455, USA

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ABSTRACT

Alcohol and nicotine are often used together, and there is a high rate of co-occurrence between alcohol and nicotine addiction. Most animal models studying alcohol and nicotine interactions have utilized passive drug administration, which may not be relevant to human co-addiction. In addition, the interactions between alcohol and nicotine in female animals have been understudied, as most studies have used male animals. To address these issues, we developed models of alcohol and nicotine co-consumption in male and female mice that utilized voluntary, oral consumption of unsweetened alcohol, nicotine and water. We first examined drug consumption and preference in single-drug, sequential alcohol and nicotine consumption tests in male and female C57BL/6 and DBA/2J mice. We then tested chronic continuous and intermittent access alcohol and nicotine co-consumption procedures. We found that male and female C57BL/6 mice readily co-consumed unsweetened alcohol and nicotine. In our continuous co-consumption procedures, we found that varying the available nicotine concentration during an alcohol abstinence period affected compensatory nicotine consumption during alcohol abstinence, and affected rebound alcohol consumption when alcohol was re-introduced. Consumption of alcohol and nicotine in an intermittent co-consumption procedure produced higher alcohol consumption levels, but not nicotine consumption levels, compared with the continuous co-consumption procedures. Finally, we found that intermittent alcohol and nicotine co-consumption resulted in physical dependence. Our data show that these voluntary co-consumption procedures can be easily performed in mice and can be used to study behavioral interactions between alcohol and nicotine consumption, which may better model human alcohol and nicotine co-addiction.

1. Introduction

Alcohol and nicotine addiction are highly co-morbid. Alcohol and nicotine are often used together, with studies showing up to 80–90% of individuals with an alcohol addiction are also smokers (Burling and Ziff, 1988; DiFranza and Guerrera, 1990; Batel et al., 1995). Alcohol and nicotine addiction are highly heritable and share common genetic factors (Swan et al., 1996; True et al., 1999) and molecular mechanisms, such as involvement of the nicotinic acetylcholine receptors (Hendrickson et al., 2013), illustrating a biological basis for alcohol and nicotine co-addiction.

Alcohol and nicotine co-use can result in complex interactions, and can produce additive effects at the behavioral (Truitt et al., 2015) and molecular level (Engle et al., 2015). For example, in men and women that use alcohol and nicotine together, alcohol itself or the presentation of alcohol cues increases the urge to smoke (Cooney et al., 2003; King et al., 2009). The interactions between alcohol and nicotine are an integral part of alcohol and nicotine co-addiction; however, most animal models are unable to capture these interactions because very few animal models employ voluntary self-administration of both alcohol and nicotine. The vast majority of animal models either examine alcohol and nicotine consumption in separate groups, which is not clinically relevant to co-addiction, or examine voluntary consumption of one drug with non-contingent administration of the other drug. Non-contingent or passive administration of drugs has been shown to result in different effects compared with voluntary administration, such as altered dopamine (Orejarena et al., 2009) and epinephrine release (Donny et al., 2000), and nicotinic receptor expression (Metaxas et al., 2010). It is possible that models using passive administration may find behavioral and pharmacological effects that are not clinically

* Corresponding author. Department of Pharmacology, University of Minnesota, 321 Church Street SE, Minneapolis, MN, 55455, USA.
E-mail address: amlee@umn.edu (A.M. Lee).

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relevant to human co-addiction. In addition, sex differences in the interactions between alcohol and nicotine consumption are understudied, as most work has been performed in male animals.

Studying alcohol and nicotine co-consumption in animals can be labor intensive and technically challenging if intravenous nicotine administration is utilized. Very few groups have performed studies of voluntary oral alcohol and intravenous nicotine self-administration in male rats (Lê et al., 2010, 2014; Scuppa et al., 2015). Due to the technical challenges of intravenous self-administration in mice, nicotine consumption is most often studied using voluntary oral consumption procedures (Klein et al., 2004; Lee and Messing, 2011; Locklear et al., 2012). Alcohol consumption has long been studied using a variety of oral consumption procedures in mice (Rhodes et al., 2005; Hwa et al., 2011; Lee et al., 2014). Mice also have the advantage of being more amenable to genetic manipulations compared with rats. However, there are no models of voluntary alcohol and nicotine co-consumption in mice.

To address these issues, we have developed several alcohol and nicotine co-consumption procedures in mice. We utilized chronic, voluntary co-consumption of alcohol and nicotine in male and female C57BL/6 and DBA/2J mice, in which the mice are presented with the choice of alcohol, nicotine and water. In this study, we first compared sequential alcohol and nicotine consumption with simultaneous co-consumption of alcohol and nicotine. In our co-consumption model, we examined the effect of alcohol abstinence and reinstatement on the consumption of both drugs. We also compared the amount of drug consumed between an intermittent and a continuous co-consumption procedure. Lastly, to determine whether chronic co-consumption of alcohol and nicotine resulted in physical dependence, we examined the development of somatic withdrawal signs in mice. Our work here shows that voluntary alcohol and nicotine co-consumption procedures can be readily performed in mice, and can be used to induce and investigate addiction-related behaviors.

2. Materials and methods

2.1. Animals and drugs

Adult male and female C57BL/6 and DBA/2J mice were a minimum of 56 days old in our experiments. Mice were purchased from Jackson Laboratory (Sacramento, CA) and acclimated to our facility for a minimum of six days before behavioral experiments. All mice were group housed in standard cages under a 12-h light/dark cycle until the start of behavioral experiments, when they were individually housed. For all experiments, food and water were freely available at all times. All animal procedures were in accordance with the Institutional Animal Care and Use Committee at the University of Minnesota, and conformed to NIH guidelines (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2010).

Alcohol (Decon Labs, King of Prussia, PA), and nicotine tartrate salt (Acros Organics, Thermo Fisher Scientific, Waltham, MA) were mixed with tap water to the concentrations reported for each experiment. The concentrations of nicotine reported are free base, and the nicotine solutions were not filtered or pH adjusted. The alcohol and nicotine solutions were not masked with any sweetener.

2.2. Experiment 1: sequential alcohol and nicotine two-bottle choice consumption tests

The objective of this experiment was to assess the levels of alcohol and nicotine consumption in C57BL/6 and DBA/2J mice when alcohol and nicotine were presented sequentially. Ten drug-naïve male and female C57BL/6 and DBA/2J mice were singly housed in double grommet cages. For the alcohol consumption portion, mice underwent a continuous 2-bottle choice alcohol consumption procedure that we have previously used (Lee et al., 2014), and were presented with a bottle of tap water and a bottle of tap water containing increasing concentrations of alcohol: 3, 6, 10, 14 and 20% v/v. Each concentration was presented for 4 days. The bottles were weighed every 2 days, and the positions of the bottles were alternated to account for side preferences. At the conclusion of the alcohol consumption portion, mice were maintained in double grommet cages and presented with only water for 2 weeks before beginning the nicotine consumption portion. Mice were presented with a bottle of tap water and a bottle of tap water containing increasing concentrations of nicotine: 30, 40 and 50 μg/mL w/v. Each concentration was presented for one week, as we have found that nicotine consumption levels stabilize within one week. The bottles were weighed every second day and the positions of the bottles were alternated to account for side preferences. All solutions were refreshed every 3–4 days. The mice were weighed once a week throughout the entire study.

2.3. Experiment 2A and 2B: co-consumption of alcohol and nicotine in a continuous access three-bottle choice test

The objective of these experiments was to assess the levels of alcohol and nicotine consumption when they were presented simultaneously, and to test the hypotheses that nicotine consumption would increase during a period of alcohol abstinence, and that re-introduction of alcohol after abstinence would result in higher levels of consumption compared with consumption prior to abstinence. For each experiment, fifteen drug-naïve male and female C57BL/6 mice were singly housed in custom cages that accommodated three drinking bottles (Ancare, Bellmore, NY). Mice were presented with three bottles containing alcohol, nicotine or water, with each set of concentrations presented for one week. Drug concentrations slowly escalated over the first three weeks before initiating the alcohol abstinence phase. The maximum nicotine concentration was 30 μg/mL to ensure adequate nicotine preference in male mice and to obtain similar nicotine consumption levels between sexes. The maximum alcohol concentration was 20% to obtain similar alcohol consumption levels between sexes. The concentrations were: for week 1–3% alcohol and 5 μg/mL nicotine, for week 2–10% alcohol and 15 μg/mL nicotine, and for week 3–20% alcohol and 30 μg/mL nicotine. The design for Experiment 2A and 2B diverged in week 4. In Experiment 2A, alcohol was removed and 30 μg/mL nicotine was presented along with the water bottle. In week 5, the 20% alcohol bottle was re-introduced along with 30 μg/mL nicotine and water. In Experiment 2B, the concentration of nicotine was dropped from 30 to 20 μg/mL during the alcohol abstinence week. This decrease in nicotine concentration was done to ensure that we did not encounter a ceiling effect for nicotine consumption and preference for the male C57BL/6 mice, since our single-drug nicotine consumption test showed that 30 μg/mL elicited the highest preference among all concentrations tested for the male C57BL/6 mice. In addition, we wanted to determine the effect of lowering the concentration of nicotine on subsequent consumption. In week 5, the 20% alcohol bottle was re-introduced, along with 20 μg/mL nicotine and water. All solutions were refreshed every 3–4 days, the weights of the bottles were measured every second day, and the mice were weighed once a week.

2.4. Experiment 3: co-consumption of alcohol and nicotine in an intermittent access three-bottle choice test

The objective of this experiment was to assess the levels of