Assessment of ethanol and nicotine interactions using a reinforcer demand modeling with grouped and individual levels of analyses in a long-access self-administration model.

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Abstract

Background: Previous reports show that both nicotine and ethanol can affect each other's rewarding and reinforcing effects but there is a lack of studies using methodological approaches that resemble the use of these substances in a vulnerable population.
Methods: Rats first self-administered ethanol, and their sensitivity to the reinforcing effects of ethanol is assessed using a reinforcer demand modeling. Rats were then equipped with intravenous catheters, allowed to self-administer nicotine, and their sensitivity to the reinforcing effects of nicotine is also assessed using a reinforcer demand modeling. In the final phase of the study, rats are allowed to self-administer ethanol and nicotine concurrently, and the effect of one substance on the rate of responding for another substance is also assessed.
Results: Our grouped assessments showed that a) ethanol was a stronger reinforcer than nicotine, b) nicotine increased self-administration of ethanol, and c) ethanol decreased self-administration of nicotine. Our individual assessments showed that a) individual demand for sucrose predicted demand for sweetened ethanol, b) individual demand for ethanol did not predict demand for nicotine, c) nicotine demand parameters predicted responding for ethanol when nicotine was available concurrently, and d) ethanol demand parameters did not predict responding for nicotine when ethanol was available concurrently.
Conclusions: Our study presents one of the ways to model ethanol and nicotine co-use and one of the ways to assess their interaction effects with the help of reinforcer demand modeling and concurrent self-administration or noncontingent administration tests.

Keywords: nicotine; ethanol; nicotine ethanol co-administration; economic demand; reinforcer demand modeling

1. Introduction

Tobacco and alcohol use are among the leading causes of preventable death in the world. Globally, tobacco and alcohol use is responsible for approximately 11 million preventable deaths (8 and 3 million, respectively; Murray et al., 2020; WHO, 2018). Furthermore, the use of these substances is highly comorbid (EMCDDA, 2009; Kohut, 2017). For example, up to 90 % of patients with alcohol use disorder also report regular tobacco consumption (Burling and Ziff, 1988; DiFranza and Guerrera, 1990; Toneatto et al., 1995). Previous reports show that combined tobacco and alcohol use is far more deleterious to users than the use of either substance alone (Frie et al., 2021; Hurt et al., 1996; Kohut, 2017; Mello et al., 1980; Mintz et al., 1985). Additional reports suggest that polydrug users may experience further challenges when seeking treatment for one of the used substances (Kozlowski et al., 1989; Stuyt, 1997).

Current treatments for substance use disorders are minimally effective, and there is a lack of individualized treatment strategies that account for polydrug history. Although assessment of group effects in research studies is vital to the general understanding of effects, assessment of average markers of performance may not provide accurate information about the mechanisms involved at different individual levels. For these reasons, there is a need to understand better the interaction between nicotine and ethanol use, focusing on individual differences.

Clinical and preclinical fields have explored the interactions between nicotine and ethanol using various methodological approaches that show contrasting effects of nicotine on alcohol reinforcement. Clinical studies show that nicotine increases alcohol consumption and how hard participants are willing to work for ethanol reinforcement (Barrett et al., 2006; Dermody et al., 2016). However, there are mixed effects in male and female human participants, with males being more sensitive to the reinforcement-enhancing effects of nicotine than females (Acheson et al., 2006; Perkins et al., 2000; for a complete review of clinical literature, see Frie et al., 2021). In comparison to clinical studies, there are varied reports regarding the effect of nicotine on alcohol reinforcement in preclinical studies. Some of the variability in the preclinical studies likely stems from variability in methodological approaches to sample relevant data. For example, some studies rely on noncontingent (bottle or vapor) ethanol delivery (Chandler et al., 2020; Lallemand et al., 2007; Marshall et al., 2003; Potthoff et al., 1983; Smith et al., 1999), while others utilize contingent operant protocols for ethanol self-administration (Barrett et al., 2020; Deehan et al., 2015; Doyon et al., 2013; Lárraga et al., 2017). Likewise, nicotine administration in those previous studies also varied with nicotine consumed noncontingently through drinking solutions (Lallemand et al., 2007; Marshall et al., 2003; Potthoff et al., 1983), contingently through drinking solutions (Deehan et al., 2015), noncontingently using systemic injections (Barrett et al., 2020; Doyon et al., 2013; Smith et al., 1999), or contingently using intravenous self-administration protocols (Chandler et al., 2020; Lárraga et al., 2017). Some of these preclinical studies show that nicotine increases the reinforcing effects of ethanol (Barrett et al., 2020; Doyon et al., 2013; Lallemand et al., 2007; Lárraga et al., 2017; Marshall et al., 2003; Smith et al., 1999) while others show no effect (Chandler et al., 2020; Deehan et al., 2015; Marshall et al., 2003). Importantly, in the two studies where nicotine and ethanol were both self-administered by rats, both substances were delivered simultaneously either in an oral solution (Deehan et al., 2015) or through an intravenous infusion (Lárraga et al., 2017). Therefore, there appears to be a significant gap in our understanding of nicotine and ethanol interactions when both substances are independently self-administered using translationally relevant routes of administration (intravenous for nicotine and oral for ethanol). There is also a significant gap in the preclinical literature investigating the interactions of nicotine and ethanol using experimental designs where both substances are available for self-administration concurrently.

Patients with alcohol use disorder are significantly more likely to smoke cigarettes than those who drink occasionally. Specifically, some estimates suggest that 80-90 % of individuals with alcohol use disorder also smoke cigarettes, and the smoking rate in this category of individuals

is significantly higher than in individuals without alcohol use disorder (Burling and Ziff, 1988; DiFranza and Guerrera, 1990; Toneatto et al., 1995). Studies show that ethanol can significantly increase cigarette smoking in individuals with alcohol use disorders, while ethanol has no effect on cigarette consumption in non-alcohol-dependent individuals (Henningfield and Goldberg, 1983; Mintz et al., 1985). Furthermore, some reports show that ethanol can increase cigarette smoking in individuals who drink alcohol regularly (4-10 drinks a week) and are moderate-to-heavy smokers (20-30 cigarettes a day; Mitchell et al., 1995). These effects of ethanol on cigarette smoking appear to be sex-dependent, as men seem more sensitive to these effects than women. For example, ethanol consumption increases the number of cigarettes smoked and the duration of smoking in men while having no effect on women's smoking patterns (King et al., 2009). In contrast to the body of literature in the clinical field detailing the effects of alcohol on cigarette smoking, there are virtually no reports on this topic in the preclinical field. Some relevant reports show that ethanol can attenuate nicotine's discriminative cue in a two-lever discrimination paradigm (Korkosz et al., 2005; although see Le Foll and Goldberg, 2005). There are also some reports showing that a combined nicotine and ethanol stimulus can produce a discriminative cue distinct from a stimulus evoked by either substance alone (Troisi et al., 2013). Altogether, previous findings suggest that ethanol may sex-dependently increase the reinforcing effects of nicotine; however, more preclinical studies are warranted to better understand the nature of this interaction.

One of the programmatic ways to assess the relationship between ethanol and nicotine reinforcement is with the help of reinforcer demand modeling. Reinforcer demand modeling is used to study behavioral responses maintained by a variety of reinforcers in both clinical and preclinical studies. The reinforcer demand approach to studying motivation for reinforcers has been adapted from the microeconomics theory, which relates the consumption of goods to the consumption expenditure (Hursh and Roma, 2016). This approach has been extensively used to assess behavioral responses for various reinforcers, including sensory stimulation, food, and drugs, to name a few (Hursh et al., 2005; Hursh and Roma, 2016, 2016). Using this approach, rats can be trained to respond for a reinforcer on a low fixed ratio (FR) schedule of reinforcement, which is then gradually increased over successive sessions, resulting in an increase of the "cost" to obtain a reinforcer. Thus, the reinforcer in this setting is conceptualized as a "good," response output maintained by the reinforcer is conceptualized as "consumption expenditure," and the FR schedule is conceptualized as "cost." Using this approach, experimenters can assess grouped and individual economic demands for the reinforcer (Kazan et al., 2020; Kazan and Charntikov, 2019; Stafford et al., 2019). The reinforcer demand modeling provides rich grouped and individual data that allows the assessment of different facets of behavior related to reinforcement. For example, various reinforcer demand models can provide demand indices that can describe demand at a price of zero (simulating free availability; Q_0), the elasticity of the demand (α), the maximum expenditure (O_{max}), and the price at which demand becomes inelastic (P_{max}). Importantly, the reinforcer demand modeling allows assessing the strength of the reinforcer represented by the degree to which the subject is willing to work for a reinforcer (*essential value* or *EV*). The main advantage of using *essential* value is that it is

a unifying measure that can be standardized across different commodities and that allows a comparison of those commodities across the reinforcement spectrum. For example, experimenters can compare the reinforcing value of heroin to the reinforcing values of cocaine, benzodiazepines, or even chicken wings (Schwartz et al., 2021). The ability to assess individual preference for different reinforcers and then apply predictive modeling to the resulting data makes reinforcer demand modeling a great candidate for a programmatic assessment of nicotine and ethanol interactions.

The current study was designed to programmatically assess nicotine and ethanol interactions in a model where both substances can be self-administered by rats using a translationally relevant long-access (12 h) self-administration model. To obtain relevant data, we thought it would be important to model extensive daily substance consumption where rats voluntarily take each substance for half of the day and then abstain from that substance for the rest of the day. We also designed this study to allow assessment of individual effects where possible, which means treating substance consumption as a continuous variable using a within-subjects design that does not require substance-abstaining controls. This approach allows assessing economic demands for various reinforcers in the same population of subjects and comparing indices derived from those demand models using predictive modeling. This approach also allows assessing whether rats with high economic demand for nicotine also demonstrate high demand for ethanol or whether certain ethanol demand indices can predict indices derived from nicotine demand. Furthermore, the indices derived from the economic demand for each reinforcer separately can then be used to predict responding for both substances simultaneously (concurrent self-administration). Importantly, the concurrent nicotine and ethanol self-administration can model conditions when one of the substances is available at a relatively low price (FR1 schedule of reinforcement) or a relatively high price (progressive ratio schedule of reinforcement; PR). Finally, the contingency for secondary substance availability can also be varied by including sessions where a secondary substance is administered noncontingently, and its effect on the primary substance is further assessed. Thus, our current study was designed to assess the ethanol and nicotine interaction in a model that closely resembles clinical use and that allows multifaceted assessment of individual data derived from the self-administration of each substance alone and the concurrent self-administration of both substances.

2. Materials

2.1. Subjects

Twenty-two male Wistar rats (250-300 g) were purchased from Envigo (Indianapolis, IN, USA). Rats were single-housed in a temperature-controlled vivarium on a 12 h light/dark cycle (lights on at 07.00). Rats were acclimated to the colony for one week before experimental procedures. Food and water were available *ad libitum* during the acclimation period and for one week after the intravenous catheter implantation surgery. Throughout the study rats were food-restricted to maintain 90 % of their free-feeding weight with water available *ad libitum*. Free-feeding weight was increased by 2 g every 30 days. All procedures were carried out in accordance with

the Guide for the Care and Use of Laboratory Animals (National Research Council et al., 2010) under review and approval of the University of New Hampshire Institutional Animal Care and Use Committee.

2.2. Apparatus

2.2.1. Self-administration chambers

Behavioral tests were carried out in Med Associates conditioning chambers measuring 30.5 × 24.1 × 21.0 cm (l × w × h) that were enclosed in a sound- and light-attenuated cubicle equipped with an exhaust fan (ENV-018MD; Med Associates, Inc.; St. Albans, VT, USA). Each chamber had aluminum sidewalls, metal rod floors, and polycarbonate for all other surfaces. Two retractable levers (147 nN required for micro-switch closure) were mounted on each side of the right-side wall. Levers were used as manipulanda to operate the retractable sipper equipped with lickometer (ENV-252M; Med Associates, Inc.; St. Albans, VT, USA) that was centered on the wall between those levers. Cue light were positioned right above each lever. For nicotine self-administration, two nosepokes were installed on the sidewall opposite from the levers. The nosepoke hole (2.5 cm in diameter) had a yellow LED mounted inside and the infrared beam monitored the entry. The infusion pump (PMH-100VS; Med Associates; St. Albans, VT, USA) for each chamber was located outside the sound-attenuating cubicle. A 5 mL syringe mounted on the infusion pump was connected with Tygon® tubing (AAQ04103; VWR; West Chester, PA, USA). The tubing was attached to a swivel coupled with a spring leash (C313C; Plastics One; Roanoke, VA, USA) which were suspended over the ceiling of the chamber on a balanced metal arm. For nicotine-alone self-administration levers and retractable sipper were removed from the chamber. Med Associates interface and software (Med-PC for Windows, version IV) were used to collect data and present programmed events.

2.2.2. Open Field

Open-field tests were conducted in an open-top square plywood box (120 cm×120 cm×25 cm; l×w×h) painted with flat black enamel. Test sessions were video recorded from a camera mounted above the apparatus and processed using ANY-maze video tracking system (Stoelting Co.; Wood Dale, IL, USA).

2.2.3. Elevated Plus-Maze

Elevated plus-maze tests were conducted using elevated plus-shaped platform (Stoelting Co.; Wood Dale, IL, USA; lane width = 10 cm, arm length = 50 cm, wall height = 40 cm, leg height = 40 cm). Test sessions were video recorded from a camera mounted above the apparatus and processed using ANY-maze video tracking system (Stoelting Co.; Wood Dale, IL, USA).

2.3. Drugs

Ethanol (200 proof; Decon Labs; King of Prussia, PA, USA) and sucrose (store bought sugar) solutions were made using tap water. Nicotine bitartrate (MP Biomedicals; Solon, OH, USA)

was dissolved in 0.9 % sterile saline. The pH of nicotine was adjusted to 7.0 ± 0.2 with a dilute NaOH solution. Nicotine doses are reported as a base. Doses and administration protocols were adopted from previous research (Charntikov et al., 2021; Kazan et al., 2020).

3. Methods

Experimental progression is shown in Figure 1. At the start of experimentation, all rats received twice daily handling for three consecutive days by all experimenters. Rats were then subjected to a baseline behavioral assessment using elevated plus-maze and open field tests. Following these baseline assessments, rats were trained to lever press for a liquid reward and were assessed for the sucrose, sweetened ethanol, ethanol-alone, and nicotine economic demands in this sequential order. Blood ethanol concentration tests and reassessment of behaviors using elevated plus-maze and open field tests occurred following assessment of ethanol-alone demand and prior to assessment of demand for nicotine. Rats then progressed to a co-administration experiment where the goal was to assess the effect of one substance on self-administration of another substance under different schedules of reinforcement and contingencies. Detailed experimental methods are presented below.

3.1. Experiment 1

3.1.1. Open field test

Rats were first acclimated to the testing room for 60 min in their home cages. Rats were then placed individually into the center of the open field apparatus for 10 min, after which they were returned to the vivarium. The open-field apparatus was divided into two portions: the center consisted of a central 60cm x 60cm square (located 30 cm from the apparatus wall), while the remaining surrounding area of the apparatus consisted of the perimeter. Total time in the center of the open field, total distance traveled, average travel speed, total number of freezing episodes, and total freezing time variables were collected using ANY-maze software. All dependent measures were divided into the first 5 min (habituation; 0-5 min) and last 5 min (test; 5-10 min) of the test. Behaviors during the second 5 min bin were used for data analyses. Open field tests were conducted prior to the lever training (see below and Figure 1) and in withdrawal from ethanol. Tests in withdrawal were performed after the acquisition of ethanol-alone economic demand (10-11 hours after the end of ethanol-alone self-administration session).

3.1.2. Elevated plus-maze test

Rats were first acclimated to the testing room for 60 min in their home cages. Rats were then placed individually into the center of the elevated plus-maze apparatus for 10 min, after which they were returned to the vivarium. ANY-maze software collected the following data: total distance traveled, average travel speed, total number of freezing episodes, total freezing time, and total time in the open arms. All dependent measures were divided into the first 5 min (habituation; 0-5 min) and last 5 min (test; 5-10 min) of the test. Behaviors during the second 5 min bin were used for data analyses. Elevated plus-maze tests were conducted prior to the lever training (see below and Figure 1) and in withdrawal from ethanol. Tests in withdrawal were

performed after the acquisition of ethanol-alone economic demand (10-11 hours after the end of ethanol-alone self-administration session).

3.1.3. Preliminary lever training

Rats were trained to drink sucrose (12 % w/v) from a retractable sipper. These sipper training sessions consisted of 120 min trials with noncontingent sucrose presentations delivered on a variable time interval (~ 3 rewards per minute). Rats were then trained to lever press for 12 % sucrose solution using the following auto-shaping procedure. At the start of each session, the house light was turned on, and a randomly selected lever (right or left) was inserted. A lever press or lapse of 15 s resulted in insertion of a sipper tube into a chamber, lever retraction, extinguishing of a house light, and illumination of both cue lights located directly above each lever. Fifteen seconds later, sipper tube was retracted, cue lights were turned off, house light was turned on, and a randomly selected lever was inserted back into the chamber. Levers were presented with the condition that the same lever could not be presented more than twice in a row, and the number of left and right lever presentations was equal across the session. Training continued until rats made lever presses on at least 80 % of lever insertions for two consecutive days (total training time was 3 to 6 daily sessions based on individual performance). One rat was removed from the study due to the inability to acquire lever-pressing behavior.

3.1.4. Acquisition of economic demand for 12 % sucrose

Rats were pseudo randomly assigned active levers with the condition that there were equal number of right and left active levers assigned. Rats were then trained to self-administer 12 % sucrose on a fixed schedule of reinforcement (FR1) for three consecutive days. Each session began with insertion of both levers and illumination of a house light. Reaching schedule requirement resulted in insertion of a sipper tube into a chamber, retraction of both levers, and illumination of cue lights. Five seconds later, sipper tube was retracted, levers were reinserted, cue lights were turned off, house light was turned back on, and rats were able to continue lever pressing for a liquid reward. Self-administration sessions were conducted during the night cycle which corresponds rodents' active phase (19.00-07.00). After 3 days of 12 % sucrose self-administration as described above, sucrose was earned on fixed ratio (FR) schedule of reinforcement that was escalated daily (between-sessions escalation) using the following sequence: 1, 3, 5, 8, 12, 18, 26, 38, 58, 86, 130, 195, 292, 438, and 657. Under this protocol, rats progressed through these daily schedule escalations until failing to earn at least one reinforcer. Subsequently, rats were allowed to self-administer 12 % sucrose on a variable schedule of reinforcement (VR3; range 1-5) until all rats completed demand assessment plus additional 3 daily sessions to reacquire 12 % sucrose self-administration.

3.1.5. Sucrose fading

Following the assessment of economic demand for 12 % sucrose, rats were trained to self-administer ethanol solution using a sucrose-fading procedure using daily 12-hour sessions. Active lever assignment remained the same from the previous phase of the experiment. Session

heuristics were identical to those described above except that over the course of the sucrose-fading phase, the liquid reinforcer was adjusted by first increasing the ethanol concentration, and then by decreasing sucrose concentration. Training began with a 12 % sucrose solution to which progressively higher ethanol concentration was gradually added every four days using the following sequence: 2 %, 4 %, 8 %, and 12 %. Rats were allowed to self-administer 12 % sucrose and 12 % ethanol solution for 6 consecutive days and then sucrose concentration was gradually decreased to 2 % using the following sequence: 12 %, 8 %, 4 %, and 2 % (four days per each concentration). At the end of this fading protocol, rats self-administered 2 % sucrose and 12 % ethanol solution using VR3 schedule of reinforcement.

3.1.6. Acquisition of economic demand for sweetened ethanol (2 % sucrose and 12 % ethanol solution)

The acquisition of economic demand for sweetened ethanol was identical to acquisition of economic demand for 12 % sucrose except that that the reinforcer was 2 % sucrose and 12 % ethanol solution. Subsequently, rats were allowed to self-administer 2 % sucrose and 12 % ethanol solution on a variable schedule of reinforcement (VR3) until all rats completed demand assessment plus additional 3 daily sessions to reacquire self-administration behavior.

3.1.7. Acquisition of economic demand for ethanol-alone

Prior to acquisition of economic demand for ethanol-alone, 2 % sucrose and 12 % ethanol solution was substituted for 12 % ethanol, active and inactive lever assignments were reversed, and cues associated with access to ethanol were changed to avoid any possible confounding effects of conditioned reinforcement associates with the sucrose reward. Thus, each ethanol-alone self-administration session began with the insertion of both levers and illumination of both cue lights located above each lever. Reaching schedule requirement resulted in insertion of a sipper tube into a chamber, retraction of both levers, turning off cue lights, and illumination of house light. Five seconds later, sipper tube was retracted, levers were reinserted, cue lights were turned on, house light was turned off, and rats were able to continue lever pressing for ethanol. Using this protocol, rats self-administered ethanol-alone on VR3 schedule of reinforcement for 7 to 10 daily 12-hour sessions until the number of active levers exceeded the number of inactive levers. Acquisition of economic demand for ethanol-alone commenced thereafter and was identical to the protocol described above except that the reinforcer was 12 % ethanol. After reaching a terminal schedule requirement, where rats fail to earn at least one reinforcer, all rats were allowed to self-administer ethanol-alone on a VR3 schedule of reinforcement until all rats completed demand assessment plus additional 3 daily sessions to reacquire ethanol self-administration. All ethanol self-administration sessions lasted 12 hours and were conducted during the night cycle (19.00-07.00).

3.1.8. Plasma ethanol concentration tests

Following the acquisition of economic demand for ethanol-alone, rats self-administered ethanol-alone on a VR3 schedule of reinforcement until completion of open field, elevated plus-maze,

and plasma ethanol concentration tests. These tests were separated by at least two daily ethanol-alone self-administration sessions. Blood ethanol concentration tests occurred immediately after one hour of ethanol-alone self-administration that substituted regular 12-hour session. There were total of two plasma alcohol concentration tests separated by at least two days of ethanol-alone self-administration. Rats were lightly restrained in a towel; the tail was placed into 46±2°C water to promote vasodilation and approximately 300 microliters blood was collected via lateral tail vein incision. The first incision was made in the distal 2 cm of the tail with subsequent incisions made at least 1 cm rostral of the previous. All samples were collected within 3 min and rats were returned to a home cage within 5 min (Drugan et al., 2005; Stafford et al., 2019). Samples were centrifuged at 4°C for 4 min at 1300 rpm to separate red blood cells and extract plasma, which was stored at -80°C until assay. Plasma alcohol concentration was measured using an Ethanol Assay Kit (ab65343; Abcam; Cambridge, UK; McCarter et al., 2017).

3.1.9. Catheter implantation surgery

Upon completion of ethanol-alone self-administration phase and all accompanied tests, rats were equipped with intravenous (IV) jugular catheters. Anesthesia was induced with 5 % isoflurane for 5 minutes and maintained at ~2.5 % for the remainder of the surgery. Butorphanol (5 mg/kg; SC) and meloxicam (0.15 mg/kg; SC) were administered after induction of anesthesia for pain management. A polyurethane catheter with a rounded tip and dual suture beads (RJVR-23; Strategic Applications Inc.; Lake Villa, IL, USA) was implanted into the right external jugular vein. The catheter was routed around the ipsilateral shoulder and affixed to a polycarbonate access port (313-000B; Plastics One Inc.; Roanoke, VA, USA) implanted along the dorsal midline 1 cm posterior to the scapulae. Immediately following surgery, catheters were flushed with 0.2 ml cefazolin (50 mg/ml) that was diluted in sterile saline with heparin (30 U/ml). This catheter flushing protocol was performed daily to maintain catheter patency throughout the self-administration phase. For the following two days after the surgery, rats were treated once a day with butorphanol (5 mg/kg; SC). After the surgery, rats were monitored daily and given at least one week to recover before proceeding to nicotine self-administration. At the end of the self-administration phase or when catheter patency loss was suspected, catheter patency was assessed using an infusion of 0.05 mL xylazine (20 mg/mL) infused through the IV catheter. This xylazine dose produces rapid and transient motor ataxia in rats with patent catheters (Kazan and Charntikov, 2019; Stafford et al., 2019). Eight rats were excluded from the study due to the loss of catheter patency throughout the study. All data from these eight rats prior to suspicion of catheter patency loss was included in the final dataset. Additional three rats did not recover from the surgery.

3.1.10. Nicotine self-administration and nicotine demand

For nicotine self-administration, chambers were reconfigured to change manipulanda from levers to nosepokes. The manipulanda were changed to minimize the conditioned enhancement of reinforcing effects associated with the manipulanda itself that were previously paired with the sucrose and ethanol stimuli. Thus, nosepokes were situated opposite of the side that previously

housed the levers. Rats spontaneously acquired nicotine self-administration using nosepokes as manipulanda. The start of each session was signaled by turning nosepoke lights on and priming the catheter with nicotine (31 µL or 90 % of internal catheter volume). Which nosepoke served as the active nosepoke was pseudo randomly assigned to ensure counterbalancing. The active nosepoke was initially reinforced using VR1.5 schedule of reinforcement (range 1-3; 3-5 days) and then using VR3 schedule of reinforcement (range 1-5; 3-5 days). Upon meeting a schedule requirement, there was a ~1 sec infusion of nicotine (0.03 mg/kg/infusion) and extinguishing of nosepoke lights for 3 sec timeout during which rats were not able to earn an infusion. All rats self-administered the exact dose of nicotine using a variation in infusion duration that was automatically calculated by the program based on their pre-session weight. All nicotine self-administration sessions lasted 12 hours and were conducted during the night cycle (19.00-07.00). Acquisition of economic demand for nicotine-alone commenced after all rats self-administered at least one nicotine infusion per session (average = 57; range 1-138, SD = 38.29). The acquisition of economic demand for nicotine-alone was identical to acquisition of economic demand for ethanol except that reinforcer was nicotine and nosepokes were used as manipulanda. After reaching a terminal schedule requirement, where rats fail to earn at least one reinforcer, all rats were allowed to self-administer nicotine-alone on a VR3 schedule of reinforcement until all rats completed demand assessment plus additional 3 daily sessions to reacquire nicotine self-administration.

3.2. Experiment 2

3.2.1. Concurrent ethanol and nicotine self-administration.

Prior to concurrent nicotine and ethanol self-administration tests all rats were assessed for baseline within session responding on the progressive ratio (PR) schedule of reinforcement. The within session PR schedule progression was identical to the between session progression used for economic demand (1, 3, 5, 8, 12, 18, 26, 38, 58, 86, 130, 195, 292, 438, and 657). Thus, rats responded for a reinforcement on a PR schedule of reinforcement for two hours and then were allowed to respond for the same reinforcer for additional ten hours on VR3 schedule. The two-hour limitation was instituted to limit learning about nonreinforcement. There were total of five ethanol and five nicotine sessions. First three sessions were conceptualized as schedule acquisition where rats gradually acclimated to the PR schedule. Therefore, only data from the last two sessions (four and five) was used to estimate the responding on the PR schedule of reinforcement for each substance.

Levers and nosepokes were available for concurrent ethanol and nicotine self-administration. All concurrent self-administration sessions lasted 12 hours. During the first two-hour portion of the concurrent session rats earned primary substance on the PR schedule of reinforcement while a secondary substance was available on FR1 or PR schedule of reinforcement. To minimize learning about non-reinforcement, during the last ten-hour portion of the session rats self-administered primary substance on a VR3 schedule while a secondary substance was available

on the FR1 schedule. Separate two-hour test sessions were conducted with a primary substance self-administered on a PR schedule of reinforcement while a secondary substance was administered noncontingently. During these separate control sessions, noncontingent nicotine infusions or access to ethanol occurred within previously defined parameters and with conjunction with previously defined associated cues. Noncontingent infusions or access to ethanol occurred every 10 min from the beginning of the session. Following these separate control sessions, all rats self-administered primary substance on a VR3 schedule of reinforcement while the secondary substance was earned on an FR1 schedule. There were total of five sessions for each testing combination. The first three sessions were conceptualized as an acclimation to the protocol and schedule conditions. Data from the last two testing sessions for each combination were used for statistical analyses. This arrangement created the following two-hour testing combinations [Primary_substance(schedule/availability)/Secondary_substance(schedule/availability)]: EtOH(PR)/Nicotine(none), Nicotine(PR)/EtOH(none), EtOH(PR)/Nicotine(FR1), Nicotine(PR)/EtOH(FR1), EtOH(PR)/Nicotine(Noncontingent), Nicotine(PR)/EtOH(Noncontingent), and EtOH(PR)/Nicotine(PR). This design allowed to sample baseline responding on PR schedule for each substance, sample responding on the PR schedule for primary substance while a secondary substance was available either at a low cost (FR1) or at relatively high cost (PR), and sample responding on the PR schedule of reinforcement while the secondary substance was administered noncontingently.

3.3. Data analysis

3.3.1. Economic demand

The assessment of economic demand for a reinforcer was based on the operant demand framework developed for indexing substance demand in clinical and preclinical fields (Gilroy et al., 2020, p. 20; Schwartz et al., 2021). Consumption data were transformed using the inverse hyperbolic sine transform (IHS, see below). This transformation is approximately log-equivalent for values of consumption greater than 5 and below 5 converges to zero, so that zero consumption values can be included in the analysis. Consumption data were processed using previously developed GraphPad Prism 8.0 template (https://ibrinc.org/behavioral-economics-tools/) and fit with the normalized version of the ZBE Model of Demand (ZBEn; Gilroy et al., 2020):

$$IHS(Q) = IHS(Q_0) * (e^{\frac{\alpha}{IHS(Q_0)} Q_0 x}) \qquad \text{(Equation 1)}$$

$$\text{where } IHS(Q_0) = log_{10}(0.5\, Q_0 + \sqrt{0.25\, Q_0^2 + 1}\,)$$

In this model, Q indicates consumption, Q_0 is consumption at 0 price, x indicates price, and α is a free parameter indicating the rate of change of the slope. Some individual data could not be fit using this model (returned as an ambiguous model fit) as their consumption at FR1 was

more than two hundred folds lower than the rest of the group. For this reason, data from one subject were removed from assessment of demand for sweetened ethanol and data from four subjects were removed from assessment of demand for ethanol-alone. In addition, data from one subject were removed from assessment of demand for nicotine-alone because it dropped out after FR1 schedule of reinforcement providing only one data point for assessment of economic demand.

The demand elasticity (α) and intensity of demand (Q_0) parameters were estimated by the model. Empirical Q_0 was calculated using amount consumed at the calculated price zero. O_{max} (maximum consumption) was calculated using the maximum consumption at each price (schedule of reinforcement). P_{max} indicates the price at which demand becomes elastic and expenditure would be maximal (O_{max}). P_{max} and O_{max} were estimated using an Excel solver tool that uses α and Q_0 parameters to solve them (https://ibrinc.org/behavioral-economi cs-tools/). Essential Value (EV), which is proportional to the inverse of α ($EV = 1/(100 \times \alpha \times \kappa^{1.5})$), was also calculated to show the rate of change in elasticity where smaller α values represent lower rate of change and higher EV or higher resistance to change responding in the face of increased cost.

All statistical analyses, unless stated otherwise, were conducted using R 4.1.3 (R Core Team, 2019). Blood ethanol concentration, variables from economic demand, and concurrent self-administration comparisons were assessed using linear mixed-effects modeling. All linear mixed-effects analyses were performed using {nlme} package for R (Pinheiro et al., 2017). The linear mixed-effects modeling approach provides a number of advantages when compared to ANOVAs. For example, this analysis does not require the assumption that the relation between the covariate and the outcome is the same across the groups and thus does not require meeting the assumption of homogeneity. Furthermore, unlike ANOVA, linear mixed-effects modeling does not assume that the different cases of data were independent and hence can model relations between different outcomes, which may be interrelated. Linear mixed-effects modeling is also more robust in dealing with missing data or unequal group sizes which is often the case in preclinical animal models. For these reasons, all relevant effects in this study were analyzed using linear mixed-effects modeling.

The relationships of demand indices across various substances and association between stress indices and demand for ethanol were assessed using simple regression analyses. The effect of withdrawal from ethanol on behavioral outcomes from the elevated plus maze and open field tests were assessed using paired samples *t*-tests. To assess how well economic demand parameters can predict responding on PR schedule of reinforcement we used backward stepwise regression that begins with a full model and at each step gradually eliminates variables from the regression model to find a reduced model that best explains the data ({MASS} package for R; Venables et al., 2002).

4. Results

4.1. Predicting blood ethanol concentration.

Volume of consumed ethanol predicted blood ethanol concentration $\left(\chi^2_{(1)} = 10.48, p = 0.0012\right)$ and explained 22 % in blood ethanol concentration variance $(R^2 = 0.22)$. The duration of contact with the sipper tube also predicted blood ethanol concentration $\left(\chi^2_{(1)} = 20.97, p < .0001\right)$ but explained almost double the amount of variance in blood ethanol concentration than ethanol volume consumed $(R^2 = 0.40)$. These results show that the duration of contact with the sipper tube is a better predictor of blood ethanol concentration than volume consumed and by extension a better predictor of ethanol consumed. Therefore, to get a more precise estimate of volume consumed during liquid self-administration sessions we used regression analysis with volume consumed as a dependent variable and the duration of contact with the sipper as a predictor. We performed this regression analysis separately for all sucrose, all sweetened ethanol, and all ethanol-alone self-administration sessions. From the resulting models we estimated the volume consumed for every second of contact with the sipper. We then converted duration of contact with the sipper to volume consumed using that estimate. We used this calculated measure of volume consumed in all our subsequent analyses to improve precision of our assessments.

4.2. Comparing demand for sucrose, sweetened ethanol, and ethanol-alone.

Demand curves for each substance are shown in Figure 2. There was a good fit of all datasets into the model (R^2 range: 0.85 - 0.97). EV differed significantly between self-administered substances $\left(\chi^2_{(3)} = 71.67, p < .0001\right)$. Essential value for sucrose was significantly higher than essential values for sweetened ethanol, ethanol-alone, or nicotine (Figure 3A). The α value differed significantly between self-administered substances $\left(\chi^2_{(3)} = 43.66, p < .0001\right)$. Nicotine α was more elastic than sucrose, sweetened ethanol, or ethanol-alone demands (Figure 3B). Q_0 value differed significantly between self-administered substances $\left(\chi^2_{(3)} = 68.56, p < .0001\right)$. Q_0 for nicotine was significantly higher than Q_0 for sweetened ethanol, ethanol-alone, or nicotine (Figure 3C). O_{max} value differed significantly between self-administered substances $\left(\chi^2_{(3)} = 70.62, p < .0001\right)$. O_{max} for sucrose was significantly higher than O_{max} for sweetened ethanol, ethanol-alone, or nicotine (Figure 3D). P_{max} value differed significantly between self-administered substances $\left(\chi^2_{(3)} = 20.54, p < .0001\right)$. P_{max} for sweetened ethanol was significantly higher than P_{max} for ethanol-alone or nicotine (Figure 3E). In addition, P_{max} for sucrose was significantly higher than P_{max} for nicotine (Figure 3E). Overall, sucrose was a strongest reinforcer (EV), when compared to sweetened ethanol, ethanol-alone, and nicotine. Sucrose also evoked highest base level of demand (Q_0) and highest level of consumption (O_{max}). On the

other hand, rats were most sensitive to changes in cost of nicotine (highest α) while showing highest peak of response (P_{max}) for sweetened ethanol.

4.3. Relationship of demand indices across economic demands.

Table 1 presents statistical output from all analyses described in this section. EV, α, O_{max}, and P_{max} of sucrose predicted the same parameter for sweetened ethanol. In other words, rats that were willing to work hard for sucrose also worked hard for sweetened ethanol. Furthermore, rats that were sensitive to changes in price of sweetened ethanol also were sensitive to changes in price of ethanol-alone (α). Interestingly, base level of demand intensity for sweetened ethanol predicted base level of demand for nicotine (Q_0).

4.4. The effect of withdrawal from ethanol on behavioral outcomes from the elevated plus maze and open field tests.

Figure 4 compares elevated plus maze and open field performance at baseline and in withdrawal from ethanol.

Elevated Plus Maze. Distance traveled, average speed, and time spend in open arms were significantly lower during withdrawal than at baseline ($t(18) = 3.03, p < 0.01$; $t(18) = 2.98, p < 0.01$; $t(18) = 2.11, p = 0.049$; respectively). Number of freezing episodes and freezing time were significantly higher in withdrawal than at baseline ($t(18) = -3.25, p < 0.01$; $t(18) = -2.24, p = 0.038$; respectively).

Open Field. Freezing time was lower during withdrawal than at baseline $t(20) = 3.34, p < 0.01$).

These findings show that elevated plus maze paradigm seems to be more sensitive in detecting behavioral withdrawal effects from ethanol.

4.5. Association between stress indices and the demand for ethanol-alone.

Individual indices from the elevated plus maze and open field tests were used to conduct simple linear regressions to assess if the demand for ethanol (the preceding self-administered substance) was predictive of withdrawal effects as assessed by these paradigms (Table 2; Independent Model). Simple regression analyses showed that economic demand for ethanol (EV) predicted behavior on four out of five elevated plus maze indices (distance traveled, average speed, number of freezing episodes, and freezing time). The economic demand for ethanol (EV) also predicted behavior on three out of five open field indices (distance traveled, average speed, and freezing time). Our findings show that economic demand for ethanol (EV) can reliably predict stress-related behavioral responses in withdrawal from ethanol.

4.6. Concurrent ethanol and nicotine self-administration.

An omnibus assessment of responding on active manipulanda for a reinforcer showed a significant effect of Condition [substance1(PR)/substance2(none), substance1(PR)/substance2(FR1), substance1(PR)/substance2(noncontingent), substance1(PR)/substance2(PR); $\chi^2_{(3)} = 8.87, p = .031$], a significant effect of substance (nicotine vs ethanol; $\chi^2_{(1)} = 99.65, p < .0001$), and their interaction $\left(\chi^2_{(3)} = 23.15, p < .0001\right)$. Thus, responding for ethanol and responding for nicotine in the presence of other substance was analyzed separately below.

The effect of ethanol on responding for nicotine. There was a significant effect of Condition [nicotine(PR)/ethanol(none), nicotine(PR)/ethanol(FR1), nicotine(PR)/ethanol(noncontingent), nicotine(PR)/ethanol(PR)] on responding for nicotine $\left(\chi^2_{(3)} = 12.40, p < 0.01\right)$. Specifically, responding for nicotine was significantly lower when ethanol was concurrently available on FR1 schedule of reinforcement in comparison to sessions when ethanol was not available [see difference between Nicotine(PR)/EtOH(none) and Nicotine(PR)/EtOH(FR1) in Figure 5A].

The effect of nicotine on responding for ethanol. There was a significant effect of Condition [ethanol(PR)/nicotine(none), ethanol(PR)/nicotine(FR1), ethanol(PR)/nicotine(noncontingent), ethanol(PR)/nicotine(PR)] on responding for ethanol $\left(\chi^2_{(3)} = 23.79, p < 0.0001\right)$. Specifically, responding for ethanol was significantly increased when nicotine was concurrently available on FR1 schedule of reinforcement or when it was available noncontingently (Figure 5B).

4.7. Economic demand parameters predicting responding on PR schedule of reinforcement.

Ethanol demand parameters predicted responding for ethanol on PR schedule of reinforcement (Table 3). Specifically, EV, α, and O_{max} explained 57, 52, and 57 % in variance (respectively) of active lever presses for ethanol-alone on PR schedule of reinforcement. On the other hand, demand parameters from the nicotine demand did not predict responding for ethanol-alone on PR schedule of reinforcement (Table 4). All nicotine demand parameters predicted responding for nicotine on PR schedule of reinforcement (R^2: 0.39-0.63; Table5). Ethanol demand parameters did not predict responding for nicotine on PR schedule of reinforcement (Table 6). Interestingly, the elasticity of nicotine demand (α) and the combination of nicotine α and O_{max} parameters predicted responding for ethanol on PR schedule of reinforcement when nicotine was available on FR1 schedule of reinforcement ($R^2 = 0.37$ and $R^2 = 0.63$ respectively; Table 7). On the other hand, nicotine demand parameters did not predict responding for ethanol on PR schedule of reinforcement when nicotine was administered noncontingently (Table 8). Finally, ethanol demand parameters did not predict responding for nicotine on PR schedule of reinforcement when ethanol was administered noncontingently (Table 9). These findings validate the use of PR schedule of reinforcement to assess individual differences in effort

allocation to self-administer nicotine or ethanol. Our findings for the first time show that nicotine demand parameters like α and O_{max} can predict responding for ethanol when it is voluntarily co-administered with nicotine. These findings further extend our understanding of dynamics between most commonly used substances like nicotine and ethanol in a closed economy setting.

5. Discussion

Nicotine and ethanol are often co-abused, and their co-administration contributes to rapid progression to dependence, adverse health consequences, and some of the highest rates of preventable mortality (Britt and Bonci, 2013, 2013; DiFranza and Guerrera, 1990; Littleton et al., 2007; Organization, 2017, 2017; Peacock et al., 2018). Previous clinical and preclinical reports show that both nicotine and ethanol can affect each other's rewarding and reinforcing effects. However, there is a lack of programmatic assessment of their interactions using methodological approaches that resemble the use of these substances in a vulnerable population. Previous preclinical studies have greatly increased our understanding of behavioral and neurobiological effects associated with ethanol and nicotine co-administration. However, while previous preclinical reports focused on the overall grouped effects in a general population, it is unclear if those effects translate to vulnerable individuals. The current study was designed to address this gap by focusing on individual differences in reinforcing effects of these two substances using within-subjects experimental design and reinforcing demand modeling as a primary reinforcement assessment tool. With this in mind, our grouped assessments show that a) ethanol is a stronger reinforcer than nicotine, b) contingent or noncontingent nicotine administration increases self-administration of ethanol, and c) contingent but not noncontingent ethanol decreases self-administration of nicotine. Our individual assessments showed that a) individual demand for sucrose predicts demand for sweetened ethanol, b) individual demand for ethanol does not predict or relate to demand for nicotine, c) nicotine demand parameters predict responding for ethanol when nicotine is available concurrently but not when nicotine is administered noncontingently, and d) ethanol demand parameters do not predict responding for nicotine when ethanol is available concurrently.

Although there is a significant preclinical research effort towards a better understanding of the etiology of substance use disorder and the development of treatment strategies, there is still a significant gap in translating that research into effective cessation and relapse prevention treatments. The effectiveness of currently available treatments is difficult to estimate because clinical studies often use different inclusion criteria and duration of observations (Alpert et al., 2013; Bottlender and Soyka, 2005; Le Foll et al., 2014; Le Strat et al., 2011; Nunes et al., 2018). For example, participants are often excluded from studies for low motivation to quit or low consumption levels (Alpert et al., 2013; Le Strat et al., 2011). With that said, the effectiveness of current treatments is marginal at best. One factor contributing to the lack of "bench to bedside" translation is the qualitatively different approach to subject selections used in clinical studies compared to grouped preclinical experimental designs. Clinical studies often recruit

individuals with an extensive history of substance use and who have high motivation to quit (e.g., those who responded to the solicitation to participate in the study). On the other hand, preclinical studies often draw their subjects from a supposedly homogeneous sample representing the general target population (e.g., outbred rodents) and then randomly assign subjects to various experimental conditions. These types of preclinical studies typically treat within-group variance as the error. One of the approaches that may improve the external validity of preclinical studies is to study the individual differences along various natural phases of the substance use continuum. The studies that focus on individual effects could provide a better understanding of prognostic and predictive markers associated with substance use disorder and can lead to treatment strategies that are more efficacious than what is currently available.

Our current study was designed to better understand individual differences in reinforcing effects of ethanol and nicotine during the drug-taking phase. Specifically, we designed a study where rats first self-administer ethanol, and their sensitivity to the reinforcing effects of ethanol is assessed using a reinforcer demand modeling. Rats are then equipped with intravenous catheters, allowed to self-administer nicotine, and their sensitivity to the reinforcing effects of nicotine is also assessed using a reinforcer demand modeling. In the final phase of the study, rats are allowed to self-administer ethanol and nicotine concurrently, and the effect of one substance on the rate of responding for another substance is assessed using a PR schedule of reinforcement. Because catheter patency usually can be maintained only for a limited period of time (30 – 60 days) and because the acquisition of ethanol self-administration using a fading protocol usually takes much longer than the acquisition of nicotine self-administration, we elected to start with an assessment of economic demand for ethanol first and then to progress to the assessment of economic demand for nicotine and subsequently to a concurrent drug administration. Because we elected to use sucrose fading for the acquisition of ethanol self-administration, this allowed us to also acquire economic demand for sucrose alone and for sweetened ethanol. Having a record of individual economic demand for sucrose alone and for sweetened ethanol allows asking deeper questions about the relationships between economic demand for a food reinforcer like sucrose, sweetened ethanol, and ethanol alone. For example, we were able to show that the economic demand for sucrose alone can largely predict the economic demand for sweetened ethanol (see EV, α, O_{max}, and P_{max} in Table 1). Specifically, our results indicate that rats that find sucrose highly reinforcing also find sweetened ethanol highly reinforcing. This finding suggests that the individual preference for food reinforcement can drive a preference for sweetened ethanol that models calorie-enriched alcoholic beverages like beer or mixed drinks. We also showed that the elasticity of demand for sweetened ethanol predicts responding for ethanol alone (see α in Table 1). This relationship suggests that individuals that show persisted responding for sweetened ethanol in the face of price increases are also insensitive to price change for ethanol alone. Importantly, because our design treats economic demand for a reinforcer as a continuous variable, we also show that rats that do not find sucrose highly reinforcing also do not find sweetened ethanol highly reinforcing and that rats that are sensitive to the price change for sweetened ethanol are also sensitive to the price

change for ethanol alone. Finally, data from this phase of the study shows that the economic demand for sucrose does not predict economic demand for ethanol-alone, suggesting that heightened sensitivity to food reinforcement does not generalize to ethanol reinforcement.

Behavioral economics stipulates that the price dictates the work output for a reinforcer. In simple terms, total consumption decreases as the reinforcer price increases (Allison, 1979; Bickel et al., 1992; Hursh, 2014). Furthermore, the relationship between consumption and price can be explained by the elasticity of economic demand, which measures the rate of consumption over the range of prices. For example, if the consumption of a reinforcer declines as the price increases, then the demand is thought to be elastic. On the other hand, if the consumption of a reinforcer remains relatively stable in the face of a price increase, then the demand is thought to be inelastic. Essential goods like bread, milk, or gasoline usually have inelastic demand because they are considered necessities, and consumers will likely purchase them despite a significant price. Addictive substances are thought to have inelastic demand as well because consumers treat them like essential goods and are often willing to pay a significant amount to obtain them (Hursh, 1984; Hursh et al., 2005; Schwartz et al., 2021). In the open economy, where a variety of goods in different categories are available on the market, the price of one commodity can affect the consumption of other commodities. In the open economy system, some commodities can substitute for others, some commodities can complement others, and some commodities are independent of others. For example, two goods are substitutes if the consumer perceives both goods as similar or comparable, decreasing consumption of one good if it has a supply of another. On the other hand, complementary good is a good whose consumption increases when the consumption of its complement increases, and the independent good is the good with no effect on the consumption of other goods. Early study investigating the relationship between ethanol and other reinforcers showed that ethanol and sucrose might function as substitutable reinforcers because constraining the availability of sucrose increases ethanol consumption (Samson and Lindberg, 1984). However, other studies showed that sweetened ethanol is more reinforcing than sucrose because pre-session feeding decreased responding maintained by sucrose but not by ethanol (Heyman, 1993), because demand for sweetened ethanol was relatively inelastic in comparison to demand for sucrose alone (Petry and Heyman, 1995), and because increasing price for a sucrose or sucrose alternatives systematically decreased their consumption while similar increases in price for sweetened ethanol did not reduce consumption in the same manner (Heyman, 2000, 1997; Heyman et al., 1999; Kim and Kearns, 2019; Petry and Heyman, 1995). Our study further extends previous reports by comparing economic demands for sucrose, sweetened ethanol, and ethanol-alone using a programmatic approach that includes main associated indexes and levels of assessment that include grouped and individual levels. Using this comprehensive approach, we show that rats were willing to work harder for sucrose than for sweetened ethanol or ethanol alone (*EV*; see Figures 2 and 3A). We also show that the demand for sucrose was more inelastic in comparison to the demand for sweetened ethanol or ethanol alone, although there were no statistically significant differences between them (see lower values of α for sucrose in Figure 3B). In contrast to previous reports discussed above, our findings suggest that sucrose is more reinforcing than sweetened ethanol and that rats are less sensitive

to price increases for sucrose than for sweetened ethanol or ethanol alone. The disagreement with previous studies likely stems from the differences in experimental design, assessment approaches, and differences in session duration between previous studies and the current study (short-access used primarily in the early studies vs. long-access employed in the present study).

We also extend previous reports that contrasted grouped reinforcing values of sucrose, sweetened ethanol, and ethanol alone by assessing individual effects associated with economic demand for these reinforcers. For the first time, we show that individual demand for sucrose can predict economic demand for sweetened ethanol. Specifically, we show that EV, α, O_{max}, and P_{max} for sucrose can all be used to predict a corresponding demand index for sweetened ethanol and that there is a positive relationship between these indices. Thus, our findings suggest that rats with high sensitivity to sucrose reinforcement (higher EV) are also highly sensitive to sweetened ethanol reinforcement. To put this into more operational terms, rats that work harder for sucrose also work hard for sweetened ethanol, and this suggests that consumption of sweetened ethanol in these individuals is driven in part by the reinforcing effects of sucrose. On the other hand, we show that the population of rats that worked harder for sweetened ethanol differs from that of rats that worked harder for ethanol alone because economic demand for sweetened ethanol did not predict responding for ethanol alone (see Table 1). It is important to note that these effects are revealed in the context of the long-access self-administration model for all of these substances. Our data suggest that in our experimental design, ethanol self-administration leads to repeated withdrawal effects that are supported by the performance on behavioral tests related to stress and anxiety (see Figure 4). Our data also shows a significant relationship between economic demand for ethanol and performance on seven out of ten measures collected from stress and anxiety-related tests, suggesting that rats with higher demand for ethanol experience a higher magnitude of withdrawal effects throughout the study (see Table 2). Overall, our findings show that on the individual level, rats that find sucrose highly reinforcing also find sweetened ethanol highly reinforcing and that this population of rats is distinct from those that find ethanol-alone highly reinforcing. Furthermore, it is possible that ethanol withdrawal effects contribute to how hard some rats are willing to work for ethanol and thus constitute another dimension of reinforcing effects associated with ethanol reward.

Previous studies used a mix of experimental approaches to assess the relationship between ethanol and nicotine use. The differences in administration models (contingent vs. noncontingent), routes of administration (drinking solutions vs. vapor vs. systemic injections vs. intravenous self-administration), and session length (short-access vs. long-access) likely contributed to the variability in reported effects. With that said, there is currently a limited understanding of the ethanol and nicotine interaction effects in the preclinical field, and that fact motivated us to design a study that would start assessing this interaction using a preclinical model with some relevancy to patterns of substance use observed in humans. For the first time, we show that rats work harder for ethanol-alone than for nicotine (see Figure 1 and 3A) and that the demand for nicotine was significantly more elastic than the demand for ethanol-alone (see Figure 1 and 3B). These findings suggest that two distinct populations of rats

with high demand for each reinforcer emerge when assessing separate economic demands for these two substances. Our interaction tests showed that both contingent "low-cost" and noncontingent nicotine administration increased responding for ethanol (see Figure 5A). It is important to point out that there was a significant difference in the effect of contingent "low-cost" and contingent "high-cost" nicotine availability on the responding for ethanol because only when nicotine was available on the FR1 schedule of reinforcement the consumption of the ethanol increased (see Figure 5A). Overall, these findings support a body of literature showing that nicotine increases the reinforcing value of ethanol (Barrett et al., 2020; Doyon et al., 2013; Lallemand et al., 2007; Lárraga et al., 2017; Marshall et al., 2003; Smith et al., 1999) with additional refinement of our understanding of the roles of nicotine contingency and "cost" in the interaction effects with ethanol. Our interaction tests also showed that contingent "low-cost" ethanol significantly decreased responding for nicotine on a progressive schedule of reinforcement while noncontingent nicotine or continent "high-cost" nicotine availability had no effect (Figure 5B). To our knowledge, this is the first demonstration of the effects of ethanol on responding for nicotine using a model where both substances are concurrently available for self-administration during the session. Altogether, our study demonstrates that it is possible to study ethanol and nicotine interaction effects using a model where both substances are self-administered separately and in a way that resembles human use (e.g., routes of administration and increased daily access). Overall, we show that the contingency and a "cost" of a co-administered substance may play an important role in the interaction effects associated with polydrug use in general or, at the very least in the interaction effects between ethanol and nicotine in particular. Additional studies will be required to confirm our effects and extend our findings toward other commonly used substances.

While it is important to understand grouped effects associated with polydrug use, there may be a significant additional benefit in investigating individual effects associated with co-administration of certain substances. One of our goals in this study was to assess individual effects associated with ethanol and nicotine co-administration. To achieve this goal, we employed the reinforcer demand modeling that allowed us to establish, prior to co-administration tests, individual demands for each reinforcer separately. In other words, with the help of the reinforcer demand modeling, we were able to determine who worked hard for the ethanol-alone and who worked hard for the nicotine reinforcement. Then, we were able to pose some important questions about individual performance during the co-administration tests. For example, we were interested in finding out if individual economic demand for each substance predicts individual performance on the PR schedule of reinforcement – a primary schedule used to assess all interaction effects. We were also interested in whether individual economic demand for each substance can predict responding during co-administration tests. We found that individual economic demand for ethanol and individual economic demand for nicotine predict individual performance on the PR schedule of reinforcement for the same substance. The confirmation that economic demand predicts performance on the PR schedule of reinforcement is important because it confirms that using the PR schedule of reinforcement, we can identify rats on the continuum from those that find each substance highly reinforcing to those that find

each substance minimally reinforcing. The ability to establish this continuum then allows us accurately assess the performance during combination tests using regression analysis. Because there are five different parameters that can be derived from each economic demand (EV, α, Q_0, O_{max}, and P_{max}), we used a conservative backward stepwise regression to determine the best model fit and the best parameter or a combination of parameters derived from the economic demand to predict the responding during combination tests. We found that the elasticity of demand for nicotine (α) on its own explains 37 % of the variance in responding for ethanol on the PR schedule of reinforcement when nicotine was also available on the FR1 schedule of reinforcement. Importantly, a combination of the elasticity of demand (α) and maximum expenditure (O_{max}) parameters explained a much larger variance (63 %) in responding for ethanol on the PR schedule of reinforcement when nicotine was also available on the FR1 schedule of reinforcement. Thus, for the first time, we are showing that it is possible to predict responding for a substance by knowing the individual performance for the companion substance (economic demand) and the mode of co-administration (schedule and contingency). We would caution readers not to draw general conclusions based on these findings because this approach and the findings derived using this multiphase study design need to be further validated with additional studies confirming the utility of this methodological strategy and the findings themselves.

Tobacco and alcohol are among the leading causes of preventable death in the world. There is evidence that their combined use may lead to a) accelerated development of dependence, b) a wider scope of negative health consequences, and c) increased difficulty in quitting one or both substances (Devlin and Henry, 2008; Frie et al., 2021; Hurt et al., 1996; Kohut, 2017; McKee and Weinberger, 2013; Mello et al., 1980; Mintz et al., 1985). Therefore, it is important to understand how these substances interact with each other under conditions that resemble substance use in a clinical population. Our study here shows one of the ways to model ethanol and nicotine co-use and one of the ways to assess their interaction effects with the help of reinforcer demand modeling and combination administration tests using PR schedule of reinforcement and noncontingent complimentary substance administration. Using this approach we were able to answer a variety of questions and able to assess data using both grouped and individual levels of assessment. One of the important findings from our study is that we show for the first time that rats willing to work harder for ethanol reinforcement are different from those willing to work harder for nicotine reinforcement. We also show for the first time that it is possible to predict the rate of responding for a substance knowing the history of economic demand for a complimentary substance during co-administration tests. Although this type of study is resource and time-demanding, we believe it is important to continue investigating polydrug use with the help of comprehensive models that can provide a broad spectrum of grouped and individual data related to different facets of polydrug use. We also believe that these types of studies that focus on individual data may benefit from a much larger sample size than what is currently practiced in the preclinical field to assess grouped effects. Additional studies focusing on individual effects associated with pharmacological interventions and on defining vulnerable endophenotypes are also needed to continue expanding our understanding of

ethanol and nicotine use comorbidity. Finally, a better understanding of how individual history of reinforcement interacts with polydrug use is urgently needed if the goal is to develop efficacious individual treatment strategies for those with multiple use disorders.

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7. References

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8. Tables

Table 1. Prediction of demand indices across economic demands.

Demand Indices		Independent Model			
Predictor	**DV**	**β**	**R^2**	**F(df)**	***p*-value**
	EV				
Sucrose	Sw.EtOH	0.62	0.38	F(1,18)=11.11	**<0.01**
Sucrose	EtOH	0.09	0.01	F(1,15)=0.13	0.72
Sucrose	Nicotine	-0.27	0.07	F(1,15)=1.16	0.30
Sw.EtOH	EtOH	0.25	0.06	F(1,15)=1.04	0.32
Sw.EtOH	Nicotine	0.05	0.00	F(1,14)=0.03	0.85
EtOH	Nicotine	0.10	0.01	F(1,11)=0.12	0.73
	α				
Sucrose	Sw.EtOH	0.86	0.74	F(1,18)=51.19	**<0.0001**
Sucrose	EtOH	0.38	0.15	F(1,15)=2.57	0.13
Sucrose	Nicotine	-0.24	0.06	F(1,15)=0.87	0.36
Sw.EtOH	EtOH	0.60	0.36	F(1,15)=8.52	**0.01**
Sw.EtOH	Nicotine	0.29	0.08	F(1,14)=1.24	0.28
EtOH	Nicotine	0.46	0.21	F(1,11)=2.99	0.11
	Q_0				
Sucrose	Sw.EtOH	0.17	0.03	F(1,18)=0.50	0.48
Sucrose	EtOH	0.04	<0.01	F(1,15)=0.02	0.88
Sucrose	Nicotine	-0.16	0.02	F(1,15)=0.38	0.54
Sw.EtOH	EtOH	-0.21	0.04	F(1,15)=0.67	0.42
Sw.EtOH	Nicotine	-0.52	0.27	F(1,14)=5.26	**0.03**
EtOH	Nicotine	-0.27	0.07	F(1,11)=0.83	0.37
	O_{max}				
Sucrose	Sw.EtOH	0.60	0.35	F(1,18)=9.87	**<0.01**
Sucrose	EtOH	0.12	0.01	F(1,15)=0.22	0.64
Sucrose	Nicotine	-0.33	0.10	F(1,15)=1.81	0.19
Sw.EtOH	EtOH	0.27	0.07	F(1,15)=1.19	0.29
Sw.EtOH	Nicotine	-0.01	<0.001	F(1,14)<0.001	0.98
EtOH	Nicotine	0.00	<0.001	F(1,11)<0.001	0.99
	P_{max}				
Sucrose	Sw.EtOH	0.55	0.30	F(1,18)=7.86	**0.01**
Sucrose	EtOH	-0.15	0.02	F(1,15)=0.32	0.57
Sucrose	Nicotine	-0.24	0.06	F(1,15)=0.90	0.35
Sw.EtOH	EtOH	0.15	0.02	F(1,15)=0.34	0.56
Sw.EtOH	Nicotine	-0.02	<0.001	F(1,14)<0.01	0.94
EtOH	Nicotine	-0.14	0.02	F(1,11)=0.21	0.65

p-values in bold indicate significant effects ($p<0.05$).

Table 2. Association between stress indices and demand for ethanol.

Stress Indices	Independent Model			
	β	**R^2**	**F(df)**	***p*-value**
EPM				
Distance Traveled	-0.50	0.25	F(1,14)=4.70	**0.048**
Average Speed	-0.50	0.25	F(1,14)=4.71	**0.048**
Freezing Episodes	0.67	0.45	F(1,14)=11.24	**<0.01**
Freezing Time	0.62	0.38	F(1,14)=8.76	**0.01**
Time in Open Arms	0.21	0.04	F(1,14)=0.64	0.44
OF				
Distance Traveled	-0.61	0.37	F(1,14)=8.38	**0.012**
Average Speed	-0.61	0.38	F(1,14)=8.46	**0.011**
Freezing Episodes	0.35	0.12	F(1,14)=1.94	0.19
Freezing Time	0.52	0.27	F(1,14)=5.18	**0.039**
Time in the Center	0.17	0.03	F(1,14)=0.44	0.52

p-values in bold indicate significant effects ($p<0.05$).

Table 3. Ethanol demand parameters predict responding for ethanol on PR schedule of reinforcement

	Dependent variable:				
	Total Active Lever Presses				
	(1)	(2)	(3)	(4)	(5)
EV	145.463**				
	p = 0.003				
alpha		-4,956.097**			
		p = 0.006			
Q0			2.394		
			p = 0.760		
Omax				7.155**	
				p = 0.003	
Pmax					1.521
					p = 0.560
Constant	21.963	250.348***	144.190*	35.827	139.943*
	p = 0.614	p = 0.00002	p = 0.044	p = 0.384	p = 0.015
Observations	13	13	13	13	13
R^2	0.572	0.520	0.009	0.569	0.032
Adjusted R^2	0.533	0.476	-0.081	0.530	-0.056
Residual Std. Error (df = 11)	78.336	82.995	119.236	78.622	117.840
F Statistic (df = 1; 11)	14.712**	11.907**	0.098	14.525**	0.363

Note: *p<0.05; **p<0.01; ***p<0.001

Table 4. Nicotine demand parameters do not predict responding for ethanol on PR schedule of reinforcement

	Dependent variable:					
	Total Active Lever Presses					
	(1)	(2)	(3)	(4)	(5)	(6)
EV	28.453					-137,619.400
	p = 0.827					p = 0.063
alpha		-198.053				
		p = 0.641				
Q0			-17,696.380			-18,346.710
			p = 0.130			p = 0.115
Omax				-1,361.757		
				p = 0.840		
Pmax					1.326	6,332.212
					p = 0.825	p = 0.063
Constant	156.683**	180.291**	230.606***	173.526**	156.587**	260.090**
	p = 0.005	p = 0.002	p = 0.0005	p = 0.007	p = 0.005	p = 0.003
Observations	14	14	14	14	14	14
R^2	0.004	0.019	0.181	0.004	0.004	0.437
Adjusted R^2	-0.079	-0.063	0.113	-0.079	-0.079	0.269
Residual Std. Error	108.888 (df = 12)	108.089 (df = 12)	98.733 (df = 12)	108.922 (df = 12)	108.882 (df = 12)	89.652 (df = 10)
F Statistic	0.050 (df = 1; 12)	0.229 (df = 1; 12)	2.657 (df = 1; 12)	0.043 (df = 1; 12)	0.052 (df = 1; 12)	2.592 (df = 3; 10)
Note:						*p<0.05; **p<0.01; ***p<0.001

Table 5. Nicotine demand parameters predict responding for nicotine on PR schedule of reinforcement

	Dependent variable:				
	Total Nosepoke Entries				
	(1)	(2)	(3)	(4)	(5)
EV	104.089*				
	p = 0.030				
alpha		-451.900**			
		p = 0.003			
Q0			-10,581.250*		
			p = 0.031		
Omax				7,566.711*	
				p = 0.022	
Pmax					4.793*
					p = 0.030
Constant	30.610	96.046***	99.609***	13.833	30.610
	p = 0.063	p = 0.00002	p = 0.0005	p = 0.480	p = 0.063
Observations	12	12	12	12	12
R^2	0.392	0.628	0.387	0.424	0.393
Adjusted R^2	0.332	0.590	0.325	0.367	0.332
Residual Std. Error (df = 10)	34.211	26.779	34.369	33.295	34.205
F Statistic (df = 1; 10)	6.456*	16.859**	6.306*	7.375*	6.462*
Note:					*p<0.05; **p<0.01; ***p<0.001

Table 6. Ethanol demand parameters do not predict responding for nicotine on PR schedule of reinforcement

	Dependent variable:					
	Total Nosepoke Entries					
	(1)	(2)	(3)	(4)	(5)	(6)
EV	7.998					-87.805
	p = 0.820					p = 0.155
alpha		-963.952				-2,855.957
		p = 0.261				p = 0.079
Q0			3.209			
			p = 0.299			
Omax				0.534		
				p = 0.730		
Pmax					-0.109	
					p = 0.923	
Constant	41.755	67.568*	23.853	40.366	49.242*	174.122*
	p = 0.197	p = 0.011	p = 0.376	p = 0.148	p = 0.031	p = 0.039
Observations	11	11	11	11	11	11
R^2	0.006	0.138	0.119	0.014	0.001	0.342
Adjusted R^2	-0.104	0.042	0.021	-0.096	-0.110	0.177
Residual Std. Error	46.820 (df = 9)	43.604 (df = 9)	44.079 (df = 9)	46.634 (df = 9)	46.937 (df = 9)	40.415 (df = 8)
F Statistic	0.055 (df = 1; 9)	1.440 (df = 1; 9)	1.216 (df = 1; 9)	0.127 (df = 1; 9)	0.010 (df = 1; 9)	2.076 (df = 2; 8)

Note: *p<0.05; **p<0.01; ***p<0.001

Table 7. Nicotine demand parameters predict responding for ethanol on PR schedule of reinforcement when nicotine is available on FR1

	Dependent variable:					
	Total Active Lever Presses					
	(1)	(2)	(3)	(4)	(5)	(6)
EV	72.658					
	p = 0.723					
alpha		-1,239.864*				-2,356.400**
		p = 0.045				p = 0.007
Q0			-34,924.210			
			p = 0.063			
Omax				4,788.596		-31,347.880*
				p = 0.739		p = 0.048
Pmax					3.338	
					p = 0.724	
Constant	264.114**	388.353***	421.642***	255.302*	264.163**	670.966***
	p = 0.006	p = 0.0002	p = 0.0004	p = 0.028	p = 0.006	p = 0.001
Observations	11	11	11	11	11	11
R^2	0.015	0.376	0.335	0.013	0.015	0.629
Adjusted R^2	-0.095	0.306	0.261	-0.097	-0.095	0.536
Residual Std. Error	162.820 (df = 9)	129.604 (df = 9)	133.754 (df = 9)	162.957 (df = 9)	162.825 (df = 9)	105.998 (df = 8)
F Statistic	0.134 (df = 1; 9)	5.416* (df = 1; 9)	4.535 (df = 1; 9)	0.119 (df = 1; 9)	0.133 (df = 1; 9)	6.776* (df = 2; 8)

Note: *p<0.05; **p<0.01; ***p<0.001

Table 8. Nicotine demand parameters do not predict responding for ethanol on PR schedule of reinforcement when nicotine is administered noncontingently

	Dependent variable:				
	Total Active Lever Presses				
	(1)	(2)	(3)	(4)	(5)
EV	42.683				
	p = 0.853				
alpha		-831.279			
		p = 0.257			
Q0			-23,930.220		
			p = 0.279		
Omax				4,756.570	
				p = 0.764	
Pmax					1.963
					p = 0.853
Constant	266.188*	349.242**	370.538**	249.538	266.205*
	p = 0.015	p = 0.003	p = 0.005	p = 0.052	p = 0.015
Observations	10	10	10	10	10
R^2	0.005	0.157	0.145	0.012	0.005
Adjusted R^2	-0.120	0.052	0.038	-0.112	-0.120
Residual Std. Error (df = 8)	179.712	165.355	166.588	179.047	179.713
F Statistic (df = 1; 8)	0.037	1.493	1.353	0.097	0.037
Note:					*p<0.05; **p<0.01; ***p<0.001

Table 9. Ethanol demand parameters do not predict responding for nicotine on PR schedule of reinforcement when ethanol is available on FR1

	Dependent variable:					
	Total Nosepoke Entries					
	(1)	(2)	(3)	(4)	(5)	(6)
EV	-18.192					1,004.113
	p = 0.554					p = 0.147
alpha		-381.876				-4,476.621
		p = 0.595				p = 0.060
Q0			3.378			2.772
			p = 0.167			p = 0.266
Omax				-0.756		-57.310
				p = 0.611		p = 0.123
Pmax					-2.842	
					p = 0.320	
Constant	51.360	41.796*	8.489	48.180	58.399*	180.245*
	p = 0.112	p = 0.037	p = 0.684	p = 0.115	p = 0.046	p = 0.049
Observations	9	9	9	9	9	9
R^2	0.052	0.043	0.254	0.039	0.141	0.770
Adjusted R^2	-0.083	-0.094	0.147	-0.098	0.018	0.541
Residual Std. Error	33.436 (df = 7)	33.607 (df = 7)	29.667 (df = 7)	33.672 (df = 7)	31.837 (df = 7)	21.772 (df = 4)
F Statistic	0.386 (df = 1; 7)	0.312 (df = 1; 7)	2.383 (df = 1; 7)	0.283 (df = 1; 7)	1.147 (df = 1; 7)	3.355 (df = 4; 4)

Note: *p<0.05; **p<0.01; ***p<0.001

9. Figures

9.1. Figure 1

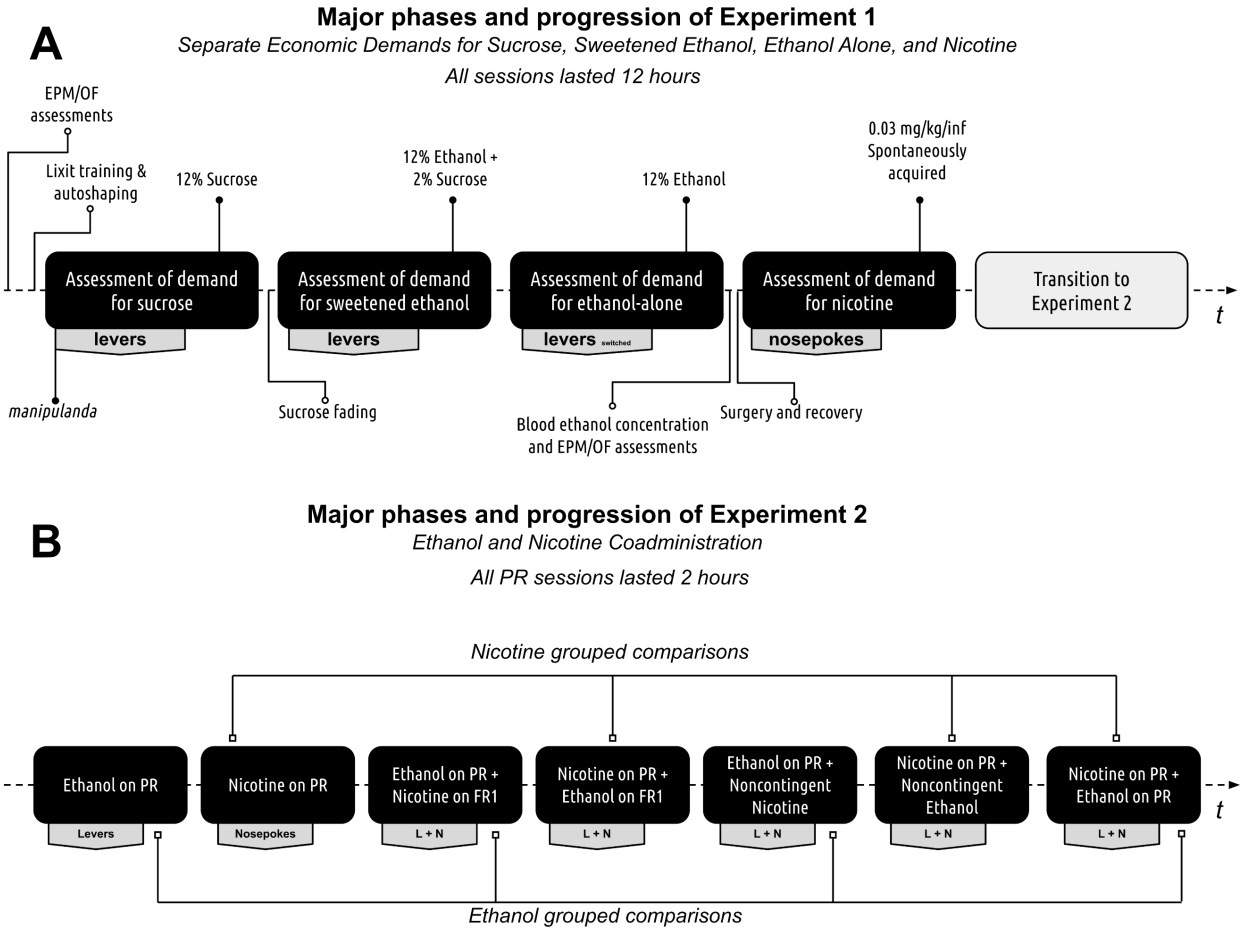

Fig. 1. Experimental progression. (A) Progression of Experiment 1. Rats were first assessed using elevated plus maze and open field tests. Rats were then trained to retrieve reward from the retractable sipper tube. Subsequently rats were trained to self-administer 12% sucrose solution, assessed for individual demand for sucrose, assessed for individual demand for sweetened ethanol, assessed for individual demand for ethanol-alone, and assessed for individual nicotine demand. Prior to nicotine self-administration phase, rats were reassessed for their performance in elevated plus maze and open field tests in withdrawal. Blood ethanol concentration was assessed at the end of the ethanol self-administration phase to confirm ethanol consumption. (B) Progression of Experiment 2. All rats were assessed for baseline responding on the PR schedule of reinforcement. Then rats were assessed for their performance during ethanol and nicotine co-administration when one substance was restricted to a PR schedule of reinforcement and the other substance was available on either FR1 schedule, PR schedule, or noncontingently.

9.2. Figure 2

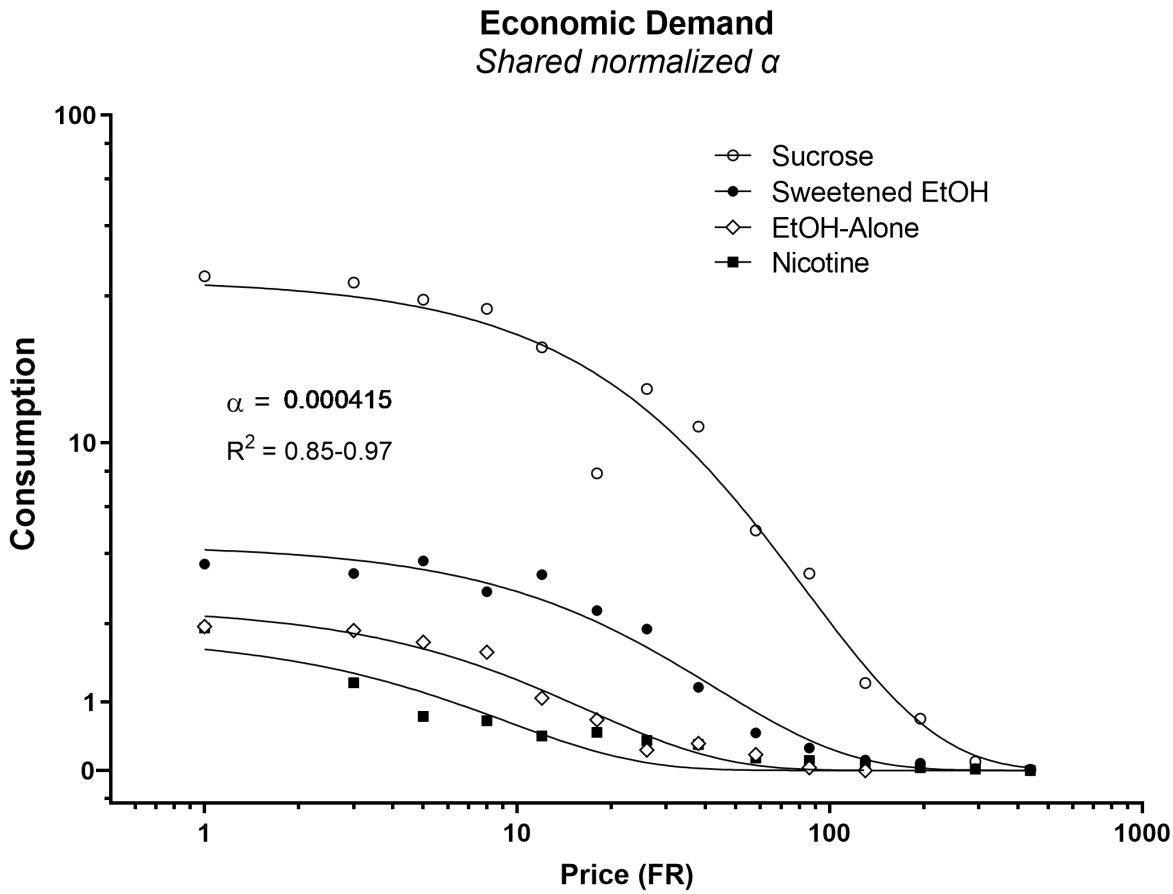

Fig. 2. Group mean demand curves for sucrose, sweetened ethanol, ethanol-alone, and nicotine. Demand curves were fit with Equation (1).

9.3. Figure 3

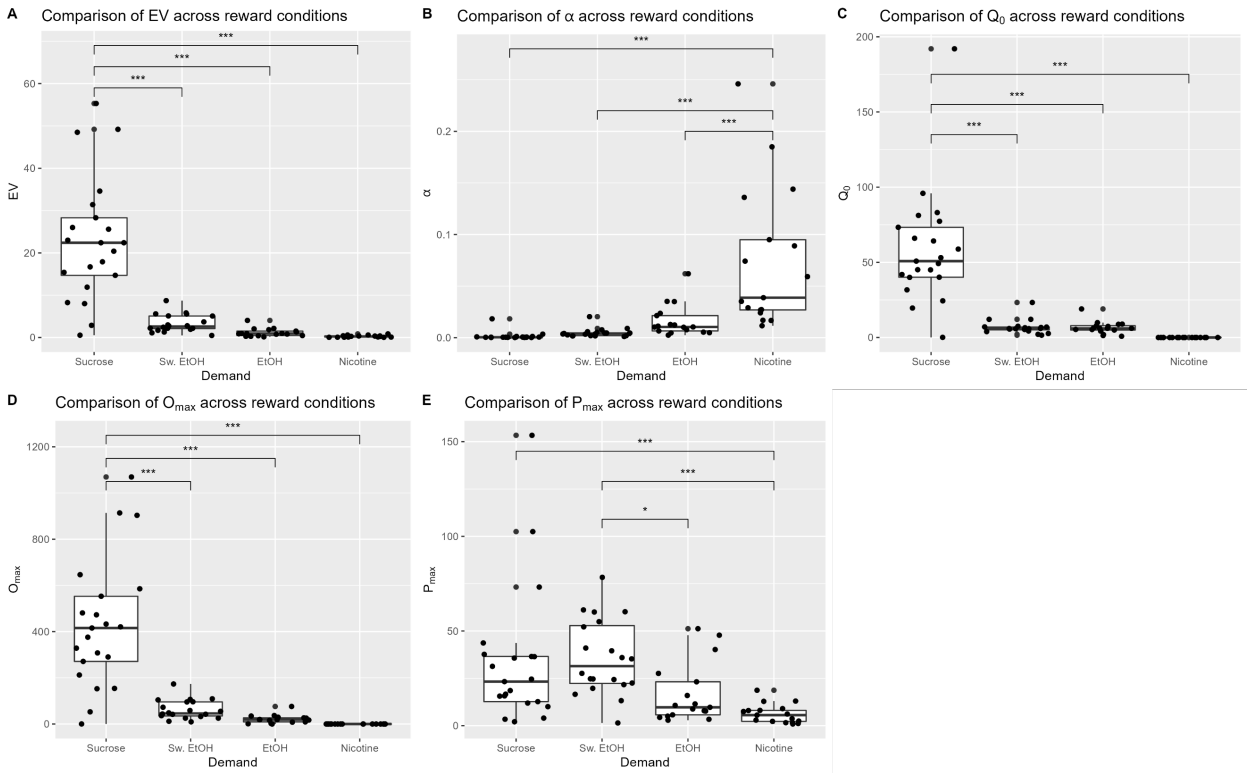

Fig. 3. Comparison of main indices derived from the reinforcer demand modeling.

9.4. Figure 4

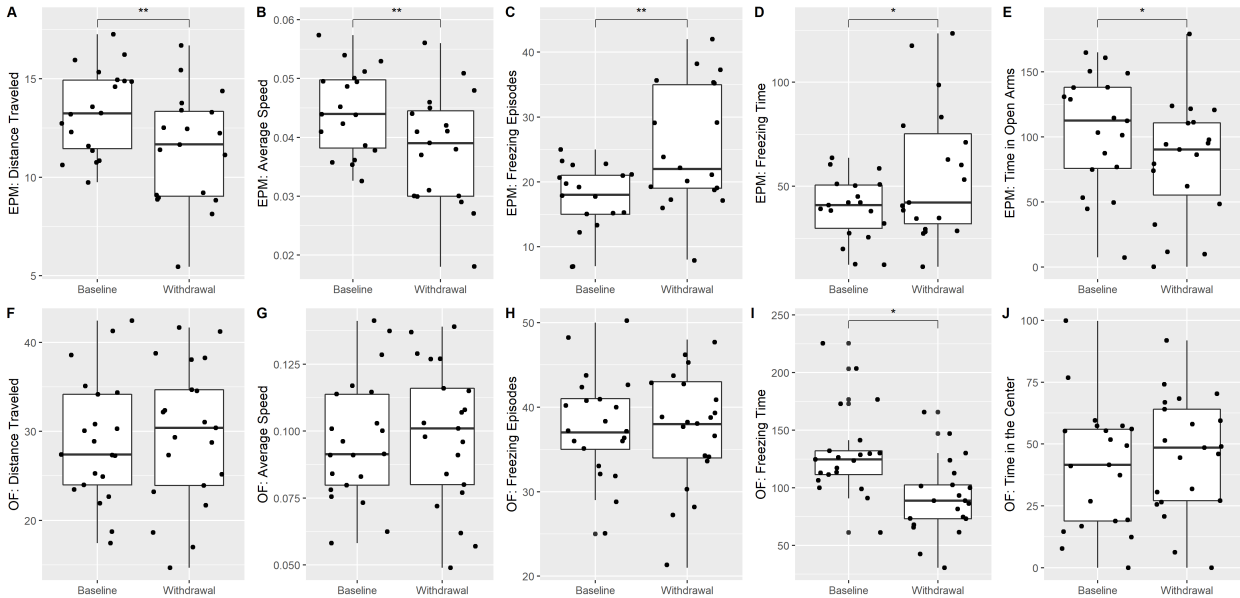

Fig. 4. The effect of withdrawal from ethanol on behavioral outcomes from the elevated plus maze (panels A-E) and open field (panels F-J) tests. Baseline tests occurred prior to administration of any substance while withdrawal tests occurred after the acquisition of ethanol-alone economic demand.

9.5. Figure 5

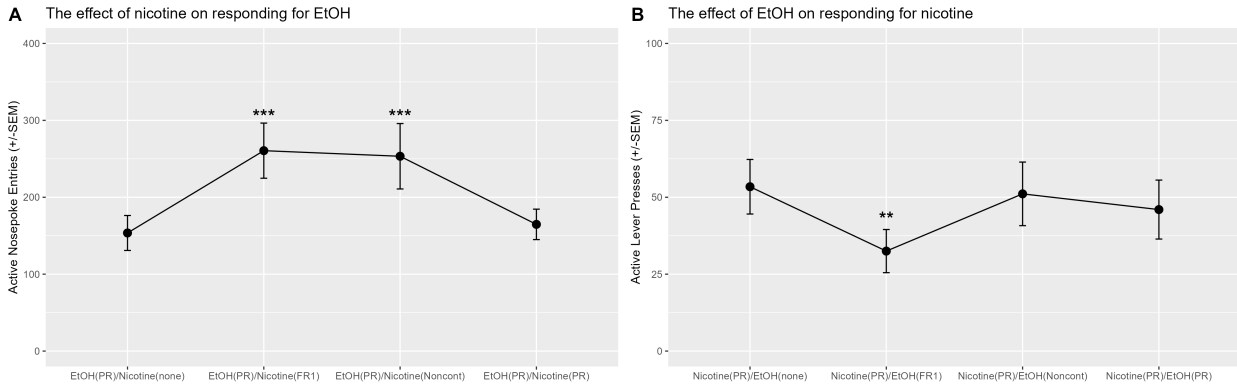

Fig. 5. (A) The effect of nicotine on responding for ethanol on PR schedule of reinforcement. Nicotine increased responding for ethanol when nicotine was available on FR1 schedule of reinforcement or noncontingently. (B) The effect of ethanol on responding for nicotine on PR schedule of reinforcement. Ethanol decreased responding for nicotine when ethanol was available on FR1 schedule of reinforcement.