Effects of naltrexone on alcohol, sucrose and saccharin binge-like drinking in C57BL/6J mice: a study with a multiple bottle choice procedure

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Abstract

Chronic alcohol (EtOH) binging has been associated with long-term neural adaptations that lead to the development of addiction. Many of the neurobiological features of EtOH abuse are shared with other forms of binging, like pathological feeding. The drinking-in-the-dark paradigm (DID) has been used extensively to study the neurobiology of EtOH binge-like drinking due to its ability to promote high intakes relevant to human behavior. DID can also generate high consumption of other tastants, but this procedure has not been fully adapted to study forms of binging behavior that are not alcohol-driven. In the present study we used a modified version of DID that uses multiple bottle availability to promote even higher levels of EtOH drinking in male C57BL/6J mice and allows a thorough investigation of tastant preferences. We assessed whether administration of systemic naltrexone (NTX) could reduce binging on EtOH, sucrose, and saccharin separately as well as in combination. Our multiple bottle DID procedure resulted in heightened levels of consumption compared to previously reported data using this task. We found that administration of the opioid receptor antagonist NTX reduced intakes of preferred, highly concentrated EtOH, sucrose, and saccharin. We also report that NTX was able to reduce overall intakes when animals were allowed to self-administer EtOH, sucrose, or saccharin in combination. Our modified DID procedure provides a novel approach to study binging behavior that extends beyond EtOH to other tastants (i.e., sucrose and artificial sweeteners), and has implications for the study of the neuropharmacology of binge drinking.

1. Introduction

Obsessive cravings and compulsive intake, often in the face of severe personal and medical consequences, are characteristic of drug abuse and binge eating disorders (Rehm et al., 2009; Sacks et al., 2015). In the case of alcohol (ethyl alcohol; EtOH), excessive drinking typically occurs in a binge-like manner (Esser et al., 2014; Kanny et al., 2018), where enough EtOH is consumed to reach high blood EtOH concentrations (BECs) in relatively short periods of time (National Institute on Alcohol Abuse and Alcoholism, 2004). Rates of binge-drinking are high, with 50-90% of all EtOH consumed taking the form of binge episodes in adults and underage adolescents, respectively (Patrick and Schulenberg, 2013; Goings et al., 2019). Binge drinking is associated with accidental injury (Gonzales et al., 2014; Stahre et al., 2014), high blood pressure (Hayibor et al., 2019), increased risk for stroke (Sundell et al., 2008), Type-2 Diabetes (Pietraszek et al., 2010), and liver dysfunction (Rosoff et al., 2019). Recurrently engaging in binge drinking may also induce long-term neuroadaptations that further promote binging and can lead to the development of addiction (Melendez, 2011; Sprow and Thiele, 2012; Carnicella et al., 2014; Tavolacci et al., 2019). Better understanding of the neurobiological consequences that arise from repeated intermittent exposure to EtOH via binging can lead to improved interventions and outcomes for addiction.

Animal models of EtOH drinking have been developed in an effort to parallel key features of human consumption. Some models have faced criticism due to lack of voluntary drinking and low levels of consumption that do not reach pharmacologically significant BECs (Rhodes et al., 2005; Thiele and Navarro, 2014). One model known as drinking-in-the-dark (DID) takes advantage of rodents' natural circadian rhythms in order to promote high levels of voluntary EtOH drinking (Rhodes et al., 2005). Using this methodology, EtOH-preferring mice consume enough EtOH to show behavioral and pharmacological signs of intoxication that are relevant to human behavior, illustrating the face validity of this procedure (Rhodes et al., 2005; Thiele and Navarro, 2014; Jeanblanc et al., 2018). Although DID has primarily been used to study the neurobiology of binge-like EtOH drinking, it can also produce elevated consumption of other drugs of abuse, including methamphetamine (Fultz et al., 2017) and opioids (Szumlinski et al., 2019) and other tastants like sugar (Kamdar et al., 2007; Cozzoli et al., 2012; Giardino and Ryabinin, 2013; Holgate et al., 2017). Although a more thorough investigation of the conditions that elicit binging on sweet tastants has not been conducted, DID's ability to promote elevated intake points at this paradigm's potential for understanding common alterations that result from binging on EtOH and other palatable rewards.

Alcohol abuse has been proposed to share many neurobiological mechanisms with other pathological forms of consumption, including high-calorie sweet foods (Avena, et al., 2012a,b; Schulte et al., 2015, 2016). A strong correlation between high levels of EtOH intake and sweet food consumption has been shown in humans (Kampov-Polevoy et al., 1999; Leggio et al., 2011). Preference for strong sweet solutions has also been shown to be associated with a paternal history of alcohol dependence (Kampov-Polevoy et al., 2001, 2003). This history of dependence has additionally been identified as a significant predictor of 'sugar-addiction' in children, suggesting a close link between alcoholism and high sweet-preference with some heritable aspects (Fortuna, 2010). Similar findings have been observed in rodents and other preclinical models. For

example, strains of mice that voluntarily consume high levels of EtOH such as C57BL/6J (B6) show higher preference indices for sucrose in comparison to other strains (Bachmanov et al., 1996, 2001). Rats characterized as 'sugar-dependent' show enhanced intake of unsweetened EtOH solutions (Avena et al., 2004). Mice sensitized to the psychomotor effects of EtOH display altered patterns of sucrose consumption, showing a more rapid initial approach and consumption of sucrose in EtOH-sensitized groups, suggesting that EtOH-induced neuroplasticity can affect consummatory behaviors for sweet rewards (Pastor et al., 2010).

EtOH and sweet tastants like sugar share chemosensory mechanisms of action (Di Lorenzo et al., 1986; Scinska et al., 2000) and significantly overlap in their actions on brain reward systems (Bodnar, 2019; Olszewski et al., 2019), including the endogenous opioid system. Rewards like EtOH and sugar may exert powerful effects on pleasure *'liking'* via coordinated actions on brain opioid receptors within a network of hedonic *'hotspots'* (Berridge and Kringelbach, 2015; Castro and Berridge, 2017). Excessive binging on sugar or EtOH, to the point of generating signs of dependence, can produce negative affective states of withdrawal that are opioid-dependent (Colantuoni et al., 2002; Avena et al., 2009; Berger et al., 2013). Opioid receptors, and in particular the mu receptor can also influence the motivational and psychomotor stimulant effects of EtOH and other reinforcers (Gianoulakis, 2001; Pastor et al., 2005, 2011; Pastor and Aragon, 2006; Kamdar et al., 2007). Pharmacological activation of mu-opioid receptors can enhance saccharin or EtOH intakes (Zhang and Kelley, 2002), while inactivation can reduce consumption of sucrose, saccharin or EtOH (June et al., 2004; Kamdar et al., 2007; Tarragón et al., 2012; Avena et al., 2014; Morales et al., 2017). The opioid receptor antagonist naltrexone (NTX), a compound with high affinity for mu-opioid receptors, is used to treat alcoholism (Volpicelli et al., 1992; Kiefer et al., 2003; Jonas et al., 2014), and in combination with bupropion (marketed as Contrave™) has recently been approved for the treatment of binge-eating disorder and weight-loss management. With B6 mice and a dose range of 2-8 mg/kg, previous data have shown that NTX can reduce EtOH binge-like intake using the DID model, although some sex differences have been reported (Kamdar et al., 2007; Tarragón et al., 2012; Zhou et al., 2019; Navarro et al., 2019). It is important to mention, however, that doses of NTX as high as 10 mg/kg have failed to reduce EtOH intake in mice selected for high blood EtOH levels (HDID lines) obtained using the DID model (Crabbe et al., 2017). HDID lines show remarkably high EtOH intakes (Crabbe et al., 2014). Higher doses of NTX might be therefore needed to reduce high binge-like EtOH intakes. Overall, although more research is needed, cumulative evidence on the effects of NTX on both EtOH and palatable food binging suggests that this compound can be a highly useful tool to explore the predictive validity of a preclinical model.

The present study was designed to investigate whether a modified multiple bottle DID paradigm could be used to study similarities in the neural alterations that result from binging on EtOH, sucrose, and saccharin. This concurrent multiple bottle (three or four bottles) procedure has several advantages over previous one and two-bottle iterations. Concurrent access to multiple bottles, whether in the same or different concentrations can produce magnified intakes (Serra et al., 2003; Tordoff and Bachmanov, 2003a; Bell et al., 2006; Rodd et al., 2009; Cozzoli et al., 2012; Colombo et al., 2014; Fultz et al., 2017), so this paradigm has the capacity to increase the likelihood of binging behav-

ior. In addition, it also allows for the investigation of the effects of various concentrations on preference and intake. We used the modified DID model to show elevated consumption of EtOH and other powerful natural reinforcers. Given that the effects of NTX have been consistently shown to reduce binge-like behavior, we administered systemic NTX in order to determine whether it could not only reduce binge-like consumption of EtOH, sucrose, and saccharin but also alter preference for various concentrations.

2. Materials and Methods

2.1 Animals

A total of 136 male C57BL/6J (B6) mice (JAX® mice strain purchased through Charles River Laboratories España S.A., Barcelona, Spain) were used in the present study. Animals were 8 weeks old at the time of arrival. They were individually housed (with no enriched environment) and kept on a standard 12-h light/dark cycle with lights on at 8:00 AM. Colony rooms were maintained at $21 \pm 1°C$ of temperature and $50 \pm 5\%$ humidity levels. Food and water were provided *ad libitum* throughout the study unless otherwise indicated. Mice were acclimated to housing and colony conditions for 2 weeks before experiment initiation. All experiments were conducted in accordance with the guidelines provided by the European Community Council Directive (2010/63/EU) for the use of laboratory animal subjects and approved by the Animal Care Committee of Universitat Jaume I.

2.2 Drugs and Drinking Solutions

EtOH, sucrose and sodium saccharin were used as drinking solutions. EtOH (99.8%) solutions (PanReac AppliChem, Barcelona, Spain) diluted to 5, 10, 20, 30 or 40% (v/v) were prepared in sterilized tap water. Sucrose solutions were prepared by dissolving $\geq 99.5\%$ sucrose (Sigma-Aldrich Química S.A. Madrid, Spain) in sterilized tap water to 5, 10, 20 or 40% (w/v). Sodium saccharine (Sigma-Aldrich, $\geq 98\%$) solutions at 0.13, 0.26, 0.53 or 1.06% (w/v) were dissolved in sterilized tap water. Concentrations of all drinking solutions were based on previous studies (Rhodes et al., 2005, 2007; Kamdar et al., 2007; Pastor et al., 2011; Tarragón et al., 2012) as well as on our own pilot studies. In the case of EtOH, a direct study of the influence of availability of different concentrations and number of tubes was included in the present study (Experiment 1). NTX hydrochloride (Sigma-Aldrich) was diluted in 0.9% physiological saline and administered intraperitoneally (IP; 10 mL/kg injection volume). The dose range of NTX (4, 8 or 16 mg/kg) used in the present study was based on previous work (Kamdar et al., 2007; Tarragón et al., 2012; Fultz et al., 2017).

2.3 Experimental Design

To evaluate binge-like drinking we used a modified version of the 4-day DID procedure introduced by Rhodes et al. (2005) in which B6 mice drink EtOH voluntarily to the point of behavioral intoxication (Rhodes et al., 2005, 2007; Kamdar et al., 2007, Lowery et al., 2010; Fultz et al., 2017). Three hours after the start of the dark cycle, wa-

ter tubes were replaced with 10 mL graduated (0.1 mL increments) cylinders with double-ball bearing sipper tubes containing different solutions depending on the experiment. For each experiment (except for Experiment 1), 4 drinking cycles (one per week) of 4 days were used. During the first 3 days of each cycle, drinking tubes were available for 2 h, then replaced with home cage water bottles. On day 4 (test day), tubes were available for 4 h, then replaced with water. Food was not removed when EtOH, sucrose or saccharin tubes were introduced. Days 1-3 provided habituation to the procedure. On day 4 (Experiments 2-6; intakes recorded at 2 and 4 h) animals received IP injections of NTX (0, 4, 8 or 16 mg/kg) 30 min before water tubes were replaced with drinking solutions. We used a counterbalanced, within-subject design; all subjects received all doses of NTX, one per week (avoiding ascending or descending dose schedules). For each cycle, habituation was conducted Tuesday-Thursday, with test on Friday. Animals were left undisturbed (with regular food/water availability) for 3 days a week; Saturday-Monday. No significant intake differences between cycles (weeks) were found. Body weights were recorded on test days. Rack-mounted empty cages with solution tubes were used as a control and correction for leakage. With double-ball bearing sipper tubes leaks were rare and minimal (i.e., 0.1 mL).

Experiment 1 evaluated DID intake using different EtOH concentrations and tube availability configurations (n = 12-15 per group). For this experiment, drinking was only assessed for one cycle. Following previous results (Cozzoli et al., 2012; Fultz et al., 2017), single and multiple tube configurations were used with different groups. EtOH 20% and 40% were tested using one single tube. Using three simultaneously available tubes, animals were offered EtOH 5%, 10% and 20%, and also EtOH 20%, 30% and 40%. Using four concurrently available tubes, animals had access to EtOH 5%, 10%, 20 and 40%. This experiment allowed us to select a multiple-tube choice version of the DID model that produced high EtOH intake.

Given that we wanted to evaluate the effects of NTX on intake and concentration preference of EtOH, sucrose or saccharin, a multiple tube choice procedure was used for all substances. The effects of NTX on EtOH, sucrose or saccharin intake were determined using 4 simultaneously available tubes. EtOH 5%, 10%, 20% and 40% (Experiment 2, n = 15), sucrose 5%, 10%, 20% and 40% (Experiment 3, n = 15) or saccharin 0.13%, 0.26%, 0.53% or 1.06% tubes (Experiment 4, n = 16) were placed using a counterbalanced order of concentrations that was changed every day to avoid prediction of tube placement. Complete ascending or descending arrangements of concentration tubes were also avoided. A separate experiment (Experiment 5; n = 9) to test the effect of NTX on water intake was conducted. This experiment followed the same design (four cycles of a 4-day DID procedure) and doses described for Experiments 1-3, except that 4 drinking tubes containing water were used. In Experiment 6 (n = 15), we were interested in evaluating the effects of NTX on intake using a procedure involving simultaneously available sucrose, saccharin and EtOH (3 tubes). Animals could choose to drink 5% sucrose, 0.13% saccharin or 20% EtOH. Given the strong preference for sweet solutions, the two lowest sucrose and saccharin solutions were used against the preferred EtOH concentration (20% EtOH; based on Experiment 2 results; mL).

2.4 Data analysis

Data were analyzed using GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA, USA). Time period and/or NTX dose were the independent variables. The dependent variables were intakes of EtOH, sucrose or saccharin (mL, g/kg) in Experiments 1-4, intake of water (mL) in Experiment 5, and intake of sucrose, saccharin plus EtOH (mL, mL/g) in Experiment 6. In Experiment 5 all tubes contained water; water intake was therefore only analyzed as total mL drunk. Due to the substantial differences in g of EtOH, sucrose or saccharin contained per mL of these substances, intakes adjusted per body weight in Experiment 6 were expressed as mL/g, instead of g/kg. As described before (Morales et al., 2017), given that we used a within-subjects pharmacological design, the effects of NTX were analyzed using repeated measures Analysis of Variance (ANOVA). Apart from time (first 2 h *vs*. second 2 h), different simultaneously available concentrations or solutions were also considered repeated factors. Experiment 1 was analyzed using a one-way ANOVA. Post-hoc comparisons were conducted using using Tukey's HSD tests. The level for significance (α) for all statistical tests was set at 0.05.

3. Results

3.1 Intake as a function of EtOH concentration and tube availability: comparison to single tube DID

Figure 1 shows EtOH intakes (g/kg; 4 h) of separate groups of animals that were offered EtOH under five different tube/concentration configurations. A one-way ANOVA [$F_{4,61} = 18.03$, $p < 0.01$] revealed a significant effect of tube condition. From the two single tube groups (A and B), those that had access to 40% EtOH showed increased intake EtOH ($p < 0.05$, compared to 20% EtOH). The two groups with the highest concentrations available (D, E), with three (20%, 30% and 40%) and four (5%, 10%, 20% and 40%) tubes respectively also showed the highest intakes ($p < 0.01$, compared to 20% EtOH). Group E was also different from group B ($p < 0.05$) and showed the highest intake during the first 2 h (data not shown) of the 4-h period. Respect to intakes per concentration in multiple tube groups, with concurrently available 5%, 10% and 20% EtOH tubes (group C), most of their intake came from the 20% tube (3.63 g/kg; 71.6% of total intake). With 20%, 30% and 40% EtOH (group D), intake from the three different tubes was similar; 2.58 g/kg from EtOH 20%, 3.03 g/kg from EtOH 30% and 2.95 g/kg from EtOH 40%, representing 30.14%, 35.40% and 34.46% of total intake, respectively. With four tubes (group E), animals obtained most of the g/kg from EtOH 20% (3.3 g/kg; 36.7%) and EtOH 40% (4.77 g/kg; 52.5%).

3.2 Effects of NTX on EtOH consumption

Table 1 shows the effects of NTX on volumes of EtOH drinking (mL). We first assessed whether NTX reduced total EtOH consumption by combining all EtOH concentrations. Over the entire 4-h period (top panel), a one-way ANOVA revealed that NTX reduced EtOH drinking [$F_{3,42} = 10.7$, $p < 0.01$]. Both 8 mg/kg ($p < 0.05$) and 16 mg/kg ($p < 0.01$) suppressed EtOH intake relative to saline, although 16 mg/kg also differed from 4 mg/kg ($p < 0.01$). We also assessed whether NTX differentially suppressed

total intakes during the first and second 2-h periods. Using a two-way ANOVA (dose x time), we found main effects of time [$F_{1,14}$ = 5.57, p < 0.05] and NTX dose [$F_{3,42}$ = 10.35, p < 0.01] with no interaction, suggesting that EtOH drinking was higher in the first 2-h period and NTX suppressed the volume of EtOH drank. We further assessed whether NTX altered drinking as a function of concentration. For the first 2 h of intake, a two-way ANOVA (concentration x dose) revealed significant effects of concentration [$F_{3,42}$ = 14.76, p < 0.01], NTX dose [$F_{3,42}$ = 7.85, p < 0.01] and an interaction between factors [$F_{9,126}$ = 2.4, p < 0.05]. Under baseline conditions, mice equally sampled between 40% and 20% EtOH, which were both preferred to the 5% option (p < 0.01). However, 8 mg/kg (p < 0.01, compared to NTX 0) and 16 mg/kg (p < 0.01, compared to 0 and 4 mg/kg) NTX treatments selectively reduced this preference by suppressing intake of the 20% solution. We found similar results over the second 2-h period, with significant effects of concentration [$F_{3,42}$ = 8.97, p < 0.01], dose [$F_{3,42}$ = 5.66, p < 0.01] and interaction between factors [$F_{9,126}$ = 3.49, p < 0.05]. However, unlike the first 2-h period, during which both 40% and 20% were sampled, 20% was the most preferred concentration of EtOH (p < 0.01 relative to 5% and 10%). The highest NTX dose abolished this preference by reducing intake of 20% EtOH (p < 0.01, compared to 0 and 4 mg/kg). Over the total 4-h period, we found main effects of concentration [$F_{3,42}$ = 16.67, p < 0.01], dose [$F_{3,42}$ = 10.7, p < 0.01] and an interaction between factors [$F_{9,126}$ = 4.38, p < 0.01]. 20% EtOH intake was preferred to 5% and 10% EtOH (p < 0.01) under baseline conditions. While 8 mg/kg NTX reduced 20% and 40% EtOH intake (compared to 0 mg/kg, p < 0.01), high NTX only suppressed 20% EtOH consumption (compared to 0 and 4 mg/kg, p < 0.01).

Figure 2 shows the effects of NTX on EtOH intake (g/kg). The same analyses described for volume intake (ml) were performed; first with all concentrations combined (panel A) and then including EtOH concentration (panels B, C, D). Figure 2A; for the first and second 2-h periods, a two-way ANOVA (time x NTX) showed main effects of time [$F_{1,14}$ = 4,62, p < 0.05] and NTX dose [$F_{3,42}$ = 8.12, p < 0.01], but no significant interaction between time and dose. An effect of NTX (one-way ANOVA) was also found for the total 4-h period [$F_{3,42}$ = 7.87, p < 0.01]. Intakes at 8 and 16 mg/kg; p < 0.01 different respect to NTX 0 mg/kg. NTX 16 mg/kg was also different from 4 mg/kg (p < 0.01). Figure 2B shows intake (as a function of EtOH concentration) during the first 2 h. A two-way ANOVA revealed significant effects of dose [$F_{3,42}$ = 9.40, p < 0.01] concentration [$F_{3,42}$ = 69.44, p < 0.01] and interaction between factors [$F_{9,126}$ = 3.71, p < 0.01]. In saline-treated animals, intakes of EtOH 20% and 40% were both higher than 5% and 10% (p < 0.01) and a difference between EtOH 20% and 40% was also found (p < 0.01). In animals treated with NTX 4 mg/kg, EtOH 20% was different from 5% (p < 0.01) and 10% (p < 0.05), and EtOH 40% was different from all other concentrations (p < 0.01). In NTX 8 mg/kg-treated groups, 20% EtOH was different from 5% (p < 0.05), and 40% EtOH was different from 5% and 10% (p < 0.01). With 16 mg/kg, 40% EtOH was different from the other three concentrations (p < 0.01). At 20% EtOH, NTX 16 mg/kg reduced intake (compared to NTX 0 mg/kg; p < 0.01). At 40% EtOH, NTX 8 mg/kg was different from 0 mg/kg and 4 mg/kg (p < 0.01), and NTX 16 mg/kg was different from 0 mg/kg (p < 0.01) and 4 mg/kg (p < 0.05). Data from the second 2-h period are shown in figure 2C; a two-way ANOVA revealed significant effects of NTX [$F_{3,42}$ = 3.28, p < 0.05] and concentration [$F_{3,42}$ = 32.01, p < 0.01]. The interaction was p =

0.058. Analysis of total intake (4-h period; figure 2D) showed significant effects of dose [$F_{3,42}$ = 7.87, $p < 0.01$], concentration [$F_{3,42}$ = 58.07, $p < 0.01$] and interaction [$F_{9,126}$ = 3.04, $p < 0.01$]. In NTX 0 mg/kg and 4 mg/kg groups, 20% and 40% EtOH were different from 5% and 10% ($p < 0.01$), and differences between 20% and 40% were also found ($p < 0.01$). In NTX 8 mg/kg groups, EtOH 40% was different from 5% and 10% ($p < 0.01$). In NTX 16 mg/kg groups, EtOH 40% was different from all other concentrations ($p < 0.01$). NTX 8 mg/kg reduced 40% EtOH intake (different from NTX 0 and 4 mg/kg; $p < 0.05$) and NTX 16 mg/kg reduced 20% EtOH intake (different from NTX 0 mg/kg; $p < 0.01$).

3.3 Effects of NTX on sucrose intake

The top panel of Table 2 shows the effects of NTX on total mL of sucrose consumed (collapsed across concentrations). A two-way ANOVA (dose x time) revealed main effects of dose [$F_{3,42}$ = 7.77, $p < 0.01$] and time [$F_{1,14}$ = 7.31, $p < 0.05$], in addition to an interaction [$F_{3,42}$ = 9.63, $p < 0.01$]. For the 4-h period, NTX also reduced volume of sucrose drinking [$F_{3,42}$ = 7.95, $p < 0.01$]. NTX 8 mg/kg reduced sucrose drinking relative to saline ($p < 0.05$), while 16 mg/kg suppressed sucrose compared to vehicle ($p < 0.01$) and 4 mg/kg ($p < 0.05$). We then assessed how NTX affected intake of the different concentrations. We found an effect of NTX [$F_{3,42}$ = 15.95, $p < 0.01$], concentration [$F_{3,42}$ = 252.80, $p < 0.01$] and dose x concentration interaction for the first 2-h [$F_{9,126}$ = 11.24, $p < 0.01$]. Under baseline conditions, 40% sucrose was preferred above the three alternatives ($p < 0.01$), and NTX selective reduced drinking of the 40% solution ($p < 0.01$). We found no effect of NTX on the second 2-h period, although there was a concentration effect [$F_{3,42}$ = 218.90, $p < 0.01$]. Finally, our analysis showed main effects of dose [$F_{3,42}$ = 6.84, $p < 0.01$], concentration [$F_{3,42}$ = 346.50, $p < 0.01$], and an interaction [$F_{9,126}$ = 4.05, $p < 0.01$] when the 0-4 h time period was analyzed. Pairwise comparisons showed that NTX 8 and 16 mg/kg reduced 40% sucrose intake ($p < 0.01$, compared to saline).

Figure 3 shows the effects of NTX on sucrose intake in g/kg. Analyses of time period effects on total intake (two-way ANOVA; figure 3A) revealed effects of time [$F_{1,14}$ = 6.12, $p < 0.05$], dose [$F_{3,42}$ = 6.58, $p < 0.01$] and an interaction between factors [$F_{3,42}$ = 8.64, $p < 0.01$]. NTX 8 and 16 mg/kg reduced intake during the first, but not second 2-h period ($p < 0.05$, compared to saline). A significant effect of NTX (one-way ANOVA) was found for the 4-h period [$F_{3,42}$ = 5.61, $p < 0.01$]. NTX 8 and 16 mg/kg were also different from saline ($p < 0.05$). Panels B, C and D represent concentration-dependent effects for 0-2 h, 2-4 h and 0-4 h periods, respectively. Concentration was a significant factor on both time periods (figures 3B and 3C; two-way ANOVA), 0-2 h [$F_{3,42}$ = 275.10, $p < 0.01$] and 2-4 h [$F_{3,42}$ = 285.80, $p < 0.01$]. However, an effect of NTX was only seen during the first 2 h [$F_{3,42}$ = 15.47, $p < 0.01$]. An interaction between factors was also found to be significant [$F_{9,126}$ = 13.49, $p < 0.01$]. NTX 8 and 16 mg/kg reduced sucrose 40% intake ($p < 0.01$ compared to saline and 4 mg/kg). Figure 3D shows 0-4 h intake; significant effects of NTX [$F_{3,42}$ = 6.67, $p < 0.01$], concentration [$F_{3,42}$ = 464.90, $p < 0.01$] and interaction [$F_{9,126}$ = 5.61, $p < 0.01$] were found. For sucrose 40%, NTX 8 and 16 mg/kg were different from saline ($p < 0.01$), and NTX 8 mg/kg was also different from NTX 4 mg/kg ($p < 0.05$).

3.4 Effects of NTX on saccharine consumption

When collapsed across concentration (mL data; Table 3), an effect of time [$F_{1,15}$ = 8.26, $p < 0.05$] and a time x dose interaction were found [$F_{3,45}$ = 5.10, $p < 0.01$]. NTX 16 mg/kg (Table 3, top section) only reduced intake during the first 2-h period ($p < 0.05$). For the total 4 h, no main effects of NTX were found. We further assessed whether NTX differentially reduced drinking of specific saccharine concentrations (dose x concentration), which is presented in the bottom panels of Table 3. Both NTX [$F_{3,45}$= 3.10, $p < 0.05$] and concentration [$F_{3,45}$ = 15.14, $p < 0.01$] influenced saccharine drinking, showing an interaction [$F_{9,135}$ = 2.21, $p < 0.05$]. Under baseline, 4 mg/kg, and 8 mg/kg NTX treatment, mice preferred the highest saccharin concentration ($p < 0.01$), consuming nearly 50% of total mL from this solution. Only the highest dose of NTX suppressed drinking of the preferred saccharine concentration ($p < 0.01$ relative to saline). We found no main effect of NTX on saccharine drinking during the second time period, despite a main effect of concentration [$F_{3,45}$ = 15.59, $p < 0.01$], and a saccharine x NTX interaction [$F_{9,135}$ = 2.35, $p < 0.05$]. Whereas the highest concentration of saccharine (1.06%) was preferred under control conditions, 16 mg/kg NTX altered this preference; it increased intake of 0.53% saccharin relative to 0 mg/kg during the second time period (2-4 h; $p < 0.01$).

Figure 4 shows the effects of NTX on saccharine intake (g/kg). Analysis of total intake data (figure 4A) including the two 2-h periods (time x dose) revealed a significant interaction between factors [$F_{3,45}$ = 5.06, $p < 0.01$]. Main effects of NTX or time were not significant (time factor effect, $p = 0.052$). NTX 16 mg/kg was different from saline (first 2-h; $p < 0.05$). Total 4-h analysis showed an effect of NTX [$F_{3,45}$ = 3.30, $p < 0.05$], with the highest dose of NTX reducing intake compared to saline ($p < 0.05$). Figures 4B, C and D represent data by concentration for the 0-2 h, 2-4 h and 0-4 h time periods, respectively. Figure 4B; a two-way ANOVA (NTX x concentration) showed significant effects of NTX [$F_{3,45}$ = 5.56, $p < 0.01$], concentration [$F_{3,45}$ = 54.66, $p < 0.01$] and interaction between factors [$F_{9,135}$ = 4.58, $p < 0.01$]. At saccharin 1.06%, NTX 8 and 16 mg/kg were found to be different from NTX 0 mg/kg ($p < 0.05$ and $p < 0.01$ respectively). For the second 2 h (figure 4C), only a main effect of concentration was found [$F_{3,45}$ = 51.19, $p < 0.01$]. Figure 4D depicts concentration-dependent effects of NTX during the total 4 h period. A two-way ANOVA (NTX x concentration) revealed an effect of concentration [$F_{3,45}$ = 56.49, $p < 0.01$] and an interaction factors [$F_{9,135}$ = 3.94, $p < 0.01$]. Post-hoc comparisons showed significant differences between NTX 0 and 16 mg/kg (saccharin 1.06%, $p < 0.01$).

3.5 Effects of NTX on water intake

NTX administration did not affect water consumption (mL). With data including the two separate 2-h periods, a two-way ANOVA (time period x NTX dose) revealed an effect of time [$F_{1,8}$ = 8.76, $p < 0.05$] but no effects of NTX or interaction between factors. NTX was also found to produce no effects on water intake when total 4-h data were analyzed. Group means (mean ml ± SEM) during the first 2 h were 0.67 ± 0.08, 0.74 ± 0.07, 0.67 ± 0.09 and 0.61 ± 0.06 (NTX 0, 4, 8 and 16 mg/kg respectively). In-

takes from the second 2 h period were 0.53 ± 0.08, 0.51 ± 0.07, 0.51 ± 0.08 and 0.56 ± 0.05 (NTX 0, 4, 8 and 16 mg/kg). Total 4 h consumption was 1.20 ± 0.11, 1.26 ± 0.12, 1.18 ± 0.10 and 1.18 ± 0.05 (NTX 0, 4, 8 and 16 mg/kg).

3.6 Effects of NTX on simultaneously available sucrose, saccharin and EtOH consumption

The top panel (Table 4) presents combined intakes from the three tubes (mL) during the two separate 2-h periods as well as total 4-h period. We found a main effect of NTX [$F_{3,42} = 7.03$, $p < 0.01$] on combined drinking but there was no time x NTX interaction. Total volume consumption over 4-h testing was reduced following administration of all NTX doses ($p < 0.01$ for NTX 4 and 8, and $p < 0.05$ for NTX 16 mg/kg). We then analyzed whether NTX treatment selectively affected drinking of the various tastants using a two-way ANOVA (dose x tastant). An effect of tastant was found for the first [$F_{2,28} = 137.10$, $p < 0.01$] and second [$F_{2,28} = 146.40$, $p < 0.01$] time periods. For the second 2-h period, an effect of NTX was also found [$F_{3,42} = 10.26$, $p < 0.01$]. No interactions between factors were found for both periods. Analysis of total 4-h intakes with the three separate tastants (dose x tastant) showed effects of tastant [$F_{2,28} = 182.70$, $p < 0.01$] and NTX [$F_{3,42} = 7.03$, $p < 0.01$], but no interaction. With data adjusted to body weight (Figure 5; ml/g), analysis of intakes combining all solutions (Figure 5A; dose x time) showed an effect of NTX [$F_{3,42} = 6.35$, $p < 0.01$], but no effects of time or dose x time interaction. Pairwise tests (0-4 h) showed that all NTX doses reduced total intake; $p < 0.05$ for NTX 4 and 16, and $p < 0.01$ for NTX 8 mg/kg. When the three different solutions were analyzed separately we found an effect of tastant for both, first [$F_{2,28} = 109.40$, $p < 0.01$] and second [$F_{2,28} = 140.10$, $p < 0.01$] 2-h periods (Figures 5B and C, respectively) but no significant interactions between NTX and tastant. An effect of NTX was also found on the second period [$F_{3,42} = 11.74$, $p < 0.01$]. Similarly, main effects of drinking solution [$F_{2,28} = 166.11$, $p < 0.01$] and NTX [$F_{3,42} = 6.35$, $p < 0.01$] were found for the total 4-h period (Figure 5D), without a significant interaction.

4. Discussion

Understanding the brain mechanisms by which vulnerable individuals develop unmanageable patterns of consumption of food or alcohol is key in order to improve treatments and prevent addiction. Over the last decade an increasing number of studies have used animal models that attempt to mimic binge intake (Thiele and Navarro, 2014; Jeanblanc et al., 2018; Treasure and Eid, 2019). Originally designed to study high voluntary EtOH consumption achieving behaviorally-intoxicating BECs, the DID model has been extensively used to investigate the neurobiology of binge-like EtOH intake (Rhodes et al., 2005; Thiele and Navarro, 2014). In the present study, we introduced a modified version of the DID procedure that further magnifies EtOH intake by the use of multiple bottles. This procedure was also shown to induce high sucrose and saccharin consumption. Our pharmacological data provide new evidence supporting a role of opioid receptors in heightened EtOH and sweet tastant intake, suggesting common neurobiological pathways that may be affected as a function of enhanced consumption.

Although there are several variations of the DID model, one of the most frequently used protocols in EtOH research involves replacing the water bottle with a tube containing 20% EtOH for 2-4 h, beginning 3 h into the dark cycle (Rhodes et al., 2005; Thiele and Navarro, 2014). With the commonly used 4-day version of this protocol, EtOH access is given over 2 h on days 1-3 but extended to a 4-h period on day 4 (test day). Using this DID protocol, different laboratories have demonstrated that genetically predisposed rodent strains such as B6 mice will drink up to ~5-7 g/kg of EtOH in 4 h (Rhodes et al., 2005; Tarragón et al., 2012; Thiele and Navarro, 2014). This level of intake produces BECs higher than 100 mg/dL and induces clear signs of behavioral intoxication evidenced by motor impairment on the rotarod and balance beam tasks (Rhodes et al., 2007). It is important to mention that the National Institute on Alcohol Abuse and Alcoholism (2004) defines binge drinking in the context of blood alcohol concentrations ($\geq$ 80 mg/dL). Given the higher rate of metabolism, minimum BECs of 100 mg/dL BECs have been proposed for mice. Interestingly, Rhodes and colleagues (2007) demonstrated that while water availability (two-bottle choice test; water vs. 20% EtOH) during a 4-h test did not reduce EtOH intake, it did affect BECs (averaging less than 80 mg/dL, as opposed to BECs greater than 120 mg/dL with access to EtOH alone). A 4-day DID protocol with a 4-h test on day 4 using a single 20% EtOH tube has therefore been extensively used to investigate the biological determinants of binge-like EtOH drinking (see Thiele and Navarro, 2014 and Jeanblanc et al., 2018 for reviews). In our laboratory we have previously used a 4-h DID procedure with a single 20% EtOH tube (Tarragón et al., 2012) achieving 5.98 $\pm$ 0.29 g/kg of EtOH (male B6 mice). This intake resulted in a range in BECs of 120-140 mg/dL, with a strong positive correlation between g/kg and BECs. Similar to what was described by Rhodes et al., (2005), we found comparable levels of EtOH intake using single 10%, 20% or 30% EtOH tubes; mL consumed across concentrations changed to achieve similar levels of g/kg (Tarragón et al., 2012).

Previous research has demonstrated that the number of bottles available during a drinking test influences EtOH intake; availability of two or more EtOH bottles of the same or different concentration increases EtOH intake in mice and rats (Spanagel et al., 1996; Serra et al., 2003; Tordoff and Bachmanov, 2003a). This phenomenon has also been described for other taste solutions such as saccharin, citric acid, quinine and sodium chloride (Tordoff and Bachmanov, 2003b). In the context of binge-like drinking, the use of protocols involving multiple concurrently available EtOH concentrations (2-4 bottles), has also been shown to produce notable increases in EtOH intake in alcohol-preferring (P) and Sardinian alcohol-preferring (sP) rats (Bell et al., 2006; Colombo et al., 2014), as well as in B6 and mixed B6 x 129X1/SvJ (B6x129) mice (Colozzi et al., 2012; Fultz et al., 2017). Similarly, Shabani et al. (2016) used selectively bred methamphetamine high drinking (MAHDR) mice to demonstrate that the availability of 3 (vs. 2 or 1) bottles of methamphetamine also increased drug intake. These studies suggest that for multiple rewards, greater availability leads to heightened consumption. Considering this evidence, we wanted to investigate whether we could boost binge-like EtOH intake with the concurrent availability of multiple EtOH bottles. Additionally, by presenting different EtOH concentrations simultaneously, we wanted to evaluate whether NTX would reduce overall EtOH consumption (Kamdar et al., 2007; Tarragón et al., 2012) or affect the pattern of EtOH intake preference across concentrations. In

preparation for this study, our data with B6 mice (Experiment 1) showed that a 4-day DID procedure (4-h test on day 4) with concurrently available 5%, 10% and 20% EtOH tubes produced an intake of 5.08 ± 0.35 g/kg of EtOH. We also tested concurrent 20%, 30% and 40% EtOH, which produced a particularly high level of EtOH intake; 8.39 ± 0.59 g/kg. In this case, intakes obtained from each tube were comparable, evidencing B6´s remarkable preference for highly concentrated EtOH solutions. In fact, with a single 40% EtOH tube animals drank up to 6.87 ± 0.48 g/kg. Favoring larger differences across concentrations (see Fultz et al., 2017), for the present study we chose a concurrent 4-bottle DID procedure with 5%, 10%, 20% and 40% EtOH. With this protocol B6 mice drank up to 9.05 ± 0.61 g/kg EtOH in 4 h (mostly from the 20% and 40% tubes); drinking approximately 60% of total intake (5.25 ± 0.42 g/kg) during the first 2 h. Compared to our own data using 4-h DID tests (current study and Tarragón et al., 2012), our present intake was > 2.5 g/kg higher than what we found with a single 20% EtOH bottle. Earlier studies using DID procedures with multiple bottles have also shown increased intake when compared to single tube 20% EtOH studies. Cozzoli et al, (2012) demonstrated that a two-bottle DID test (concurrent 5% and 20% EtOH) produced intakes of ~5 g/kg (2 h) in B6x129 mice. Particularly relevant for the present study are the data presented by Fultz et al. (2017) with the same four concentrations used here; they showed impressive 2-h DID intakes of ~6 g/kg in B6 and ~7.5 g/kg in B6x129 male mice in 2 h. Altogether, these data indicate that multiple bottle choice DID procedures can produce particularly high EtOH intakes. Whether this high drinking behavior is associated with availability of different concentrations, the number of tubes that are presented, or both, will need to be elucidated in future experiments.

It is important to mention that in the present study we did not measure BECs. However, given our previous data (Tarragón et al., 2012) and those published by others (see Crabbe et al., 2011), we can definitely suggest that the intakes of EtOH seen here would be expected to exceed 100 mg/dL. It is also relevant to point out that our data reflect intakes averaged over a period of 4 weeks (four, 4-day cycles; 4 days of EtOH availability followed by 3 days with no EtOH exposure). It could be argued that the intakes reported here were influenced by escalated drinking over repeated cycles of drinking and withdrawal. Although this effect has been previously reported in B6 (Fultz et al., 2017), and a trend was seen in our data, we did not find significant differences in overall EtOH intake across weeks 1-4 (8.34 ± 1.04 and 9.74 ± 1.32 g/kg for weeks 1 and 4, respectively; t-test, $p > 0.05$). It might be possible that longer periods of time are required to find clear time-dependent differences. Wilcox et al., (2014) found changes in EtOH consumption when DID was extended over a period of 6 weeks; intakes of weeks 4-6 were higher than those recorded during weeks 1-3. Additionally, this study revealed an interesting pattern of EtOH intake (described as 'front-loading' behavior) characterized by an increased rate of consumption during the first 15 min of the drinking session. The amount of EtOH drunk during the first part (first 30 min) of the session was also found to double from week 1 to week 6, indicating that this phenomenon developed over time (Wilcox et al., 2014). The use of repeated cycles of DID including multiple EtOH bottles might be an interesting strategy to boost binge-level drinking in future studies.

The predictive validity of DID as an animal model has been tested using drugs approved to treat alcoholism, including the opioid receptor antagonist naltrexone

(NTX). NTX is known for its efficacy in reducing high alcohol drinking in relapsing patients (Volpicelli et al., 1992; Kiefer et al., 2003; Jonas et al., 2014). NTX, alone or in combination with other drugs, has been shown to reduce EtOH intake using DID methodology (Kamdar et al., 2007; Tarragón et al., 2012; Ripley et al., 2015; Zhou et al., 2019; Navarro et al., 2019). These results add to an extensive list of studies indicating that NTX reduces EtOH consumption using a variety of methodologies and animal models (Davidson and Amit, 1997; Sharpe and Samson 2001; Fachin-Scheit et al., 2006), including genetically predisposed EtOH-preferring strains of rodents such as B6 mice (Le et al., 1993, Phillips et al., 1997; Middaugh et al., 2003; Dhaher et al., 2012). The current study presents new evidence showing that high binge-like EtOH drinking can be reduced by NTX. In particular, a reduction in intake was seen at the doses of NTX 8 and 16 mg/kg, but not 4 mg/kg. We have previously shown reductions in EtOH drinking with NTX 4 mg/kg using DID tests with a single 20% EtOH tube (Tarragón et al., 2012), an effect also found by Kamdar and colleagues (2007) with NTX 2 mg/kg. The particularly high intake of EtOH found with our procedure may be associated with the fact that higher doses of NTX were needed to reduce EtOH consumption. Consistent with this, Crabbe et al. (2017) showed that NTX 10 mg/kg failed to reduce EtOH drinking in mice selectively bred for their high BECs using a DID procedure (HDID mice), which also showed high EtOH intake. Additionally, we observed a stronger, dose-dependent NTX effect during the first 2 h; NTX reduced consumption of those concentrations that capitalized intake (EtOH 20% and 40%). However, during the second 2 h, an increased preference for the highest EtOH concentration diminished the overall effect of NTX. As a result, for the total 4-h period, a reduced 20%, but not 40% EtOH intake was found with NTX 16 mg/kg. By increasing intake from the most pharmacologically efficient EtOH tube (40%), B6 mice might have tried to oppose the reward-reducing effects of a high NTX dose. Overall, the present DID data support extensive evidence proposing a role of the endogenous opioid system in the neurobiology that mediates EtOH drinking (Gianoulakis, 2001; Font et al., 2013). Future studies will need to elucidate the involvement of mu-, delta- and/or kappa-opioid receptors using EtOH DID protocols. Mice lacking the pro-opiomelanocortin (POMc)-derived peptide beta-endorphin showed reduced DID using 7.5%, 15% or 30% EtOH solutions, suggesting a pivotal role of mu- and delta-opioid receptors (Zhou et al., 2017a).

The endogenous opioid system has also been linked to sugar and other sweet rewards (Kelley et al., 2002; Olszewski and Levine, 2007; Berridge and Kringelbach, 2015). Intake of saccharin solutions increases by infusions of a mu opioid agonist into the ventral striatum (Zhang and Kelley, 2002), and pharmacological antagonism of opioid receptors can reduce consumption of sucrose and saccharin (Biggs and Myers, 1998; June et al., 2004; Morales et al., 2017). Also, excessive sugar intake can produce withdrawal-like symptoms associated with alterations in opioid receptor signaling (Colantuoni et al., 2002; Avena et al., 2009). In our study, B6 mice showed remarkably high intakes of sucrose, drinking up to 32.75 ± 1.40 g/kg in 4 h (0-2 h; 19.5 ± 1.39 g/kg, and 2-4 h; 13.25 ± 1.10 g/kg). Our previous data using a 2-h 10% sucrose DID test showed that B6 mice consumed ~6.8-8.2 g/kg (Tarragón et al., 2012). In our current study, intake (both in mL and g/kg) was obtained almost exclusively from the 40% tube. Our pilot studies (data not shown) revealed that, regardless of the combination of concentrations, mice always obtained most of their intake from the highest sucrose concentration

available. Due to extreme solution viscosity we did not use concentrations higher than 40%. With our procedure, we found that the two highest doses of NTX (8 and 16 mg/kg) reduced sucrose drinking, an effect mostly seen during the first 2 h of the 4-h test. Studies using less concentrated solutions (i.e, 10%) and lower doses of NTX have reported no effects of this antagonist on DID sucrose consumption (Kamdar et al., 2007; Tarragón et al., 2012). Future studies will need to clarify whether, as the current data suggest, the effect of NTX on sucrose DID requires higher levels of binge-like intake and/or higher doses of NTX.

High saccharin intake was also achieved with our multiple-bottle DID protocol, reaching 0.72 ± 0.05 g/kg in 4 h (0-2 h; 0.42 ± 0.04 g/kg, and 2-4 h; 0.30 ± 0.03 g/kg). This intake was significantly higher than what has been previously reported with other DID experiments that used lower concentrations. Using 0.1% saccharin, Zhou et al. (2019) found intakes around 0.1 g/kg in 4 h (male B6), while Kamens et al. (2018) showed drinking up to ~0.02 g/kg in 2 h (adolescent male and female B6; 0.033% saccharin). Our animals preferred the concentration of 1.06%. However, this preference for the highest concentration was not as skewed as that observed with sucrose. Approximately 50% and 20% of total intake (mL) was obtained from the 1.06% and 0.53% tubes, respectively. To the best of our knowledge, the current study presents the first data showing that NTX reduces saccharin intake using a DID procedure. A combination of bupropion 10 mg/kg + NTX 1 mg/kg, capable of reducing 4% sucrose intake, did not affect 0.1% saccharin DID consumption (Zhou et al., 2019). However, previous research using non-DID procedures has found that opioid receptor antagonism can reduce saccharin intake (Lynch and Libby, 1983; Chow et al., 1997; Biggs and Myers, 1998). In our case, high saccharin DID was particularly resistant to the effects of NTX; we had to administer 16 mg/kg of NTX to see a reduction in overall intake. Similar to what we found with sucrose, this effect was mostly observed during the first 2 h. Concentration-dependent analyses showed that NTX 8 mg/kg also reduced intake of 1.06% during the first 2 h. Interestingly, during the 2-4 h period, NTX 16 mg/kg increased intake of saccharin 0.53% (in mL) and did not reduce 1.06% drinking. This effect mirrors our findings in the EtOH study and might reflect compensatory mechanisms aimed to outweigh the effects of NTX.

One advantage of multiple-bottle drinking procedures is that they can also be used to assess intake preferences across different solutions. In the present study we investigated consumption of low concentrations of sucrose (5%) and saccharin (0.13%) concurrently available with 20% EtOH. Mice preferred sweet solutions over EtOH, overall consuming most from the saccharine option, followed by sucrose. In comparison to our single tastant studies, total intakes were significantly higher for this experiment, with values exceeding 4.5 mL in 4 h. Perhaps the fact that sucrose and saccharin were offered at lower concentrations allowed them to drink more volume. This elevated drinking prompted us to examine water intake during the remaining 20 h (non-experimental phase), finding that mice consumed ~90% of their daily fluid intake during the 4-h DID test (data not shown). It is also interesting to point out that, with this combination of solutions, total intakes during the first and second 2-h periods were comparable. This differs from our single solution experiments where animals tended to 'front-load' intakes in the first 2 h period. In addition, the effect of NTX on intake with three different solutions was also different. We found that all NTX doses, including 4 mg/kg, re-

duced intake (overall 4-h drinking), and this effect was particularly significant during the second 2 h of the test. Sucrose and saccharin intakes were not differentially affected by NTX. This is important to highlight because this experiment was originally designed to evaluate whether NTX would produce differential effects on intake when animals can choose between caloric (EtOH, sucrose) and non-caloric (saccharin) solutions. Our data suggest that both, taste and post-ingestive aspects of sucrose and saccharin intake can be regulated by opioid receptors. It has been argued that homeostatic factors can affect DID (Tabarin et al. 2007; Thiele and Navarro, 2014). However, manipulations to the homeostatic state of animals, either by food deprivation or pharmacological manipulations of molecules that influence appetite have all failed to alter the high levels of EtOH intake produced by the DID procedure (Lyons et al., 2008). This suggests that such levels of consumption are likely to be driven by the reinforcing pharmacological effects of EtOH. The ability of NTX to reduce intake is therefore likely due to its ability to interfere with such reinforcing properties of EtOH. Future experiments with food-deprived and sated animals might help clarify the role of the opioid system in modulating sucrose and saccharin binge-like drinking using DID methodology.

Concluding remarks and future directions

Drug abuse and binge eating disorders share imbalances in brain systems that regulate motivation, reward saliency, decision-making, and self-control (Volkow et al., 2017; Wiss et al., 2018). Together with mesolimbic dopamine, the endogenous opioid system has been shown to play an important role in regulating food and drug reward (Volkow et al., 2013, 2017; Berridge and Kringelbach, 2015). Clinical evidence indicates that endogenous opioids promote intake of sweet and fat tastants, and a polymorphism of the mu opioid receptor gene has been associated with binge eating disorders (Davis et al., 2009; Olszewski et al., 2011; Volkow et al., 2013, 2017). In the current study we introduced a modified version of an animal model of binge-like intake (DID) that produces notably high consumption of EtOH, sucrose and saccharin, and present evidence of an involvement of opioid receptors in such intakes. This high binge-like intake-promoting procedure expands our methodological options to investigate overconsumption of palatable foods and addictive substances. In future studies, it could be used to investigate the role of sex, energy homeostasis, history of drug experience as well as to test new pharmacological tools in the context of binge intake research.

5. Acknowledgements

6. References

Avena NM, Carrillo CA, Needham L, Leibowitz SF, Hoebel BG (2004). Sugar-dependent rats show enhanced intake of unsweetened ethanol. *Alcohol* **34**: 203–9.

Avena NM, Rada P, Hoebel BG (2009). Sugar and fat binging have notable differences in addictive-like behavior. *J Nutri* 139: **623**–8.

Avena NM, Gearhardt, AN, Gold MS, Wang GJ, Potenza, MN (2012a). Tossing the baby out with the bathwater after a brief rinse? The potential downside of dismissing food addiction based on limited data. *Nat Rev Neurosci* **13**:*514.*

Avena NM, Gold JA, Kroll C, Gold MS (2012b). Further developments in the neurobiology of food and addiction: update on the state of the science. *Nutrition* **28**: 341–3.

Avena NM, Bocarsly ME, Murray S, Gold MS (2014). Effects of baclofen and naltrexone, alone and in combination, on the consumption of palatable food in male rats. *Exp Clin Psychopharmacol* **22**: 460–7.

Bachmanov AA, Tordoff MG, Beauchamp GK (1996). Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. *Alcohol Clin Exp Res* **20**: 201–6.

Bachmanov AA, Tordoff MG, Beauchamp GK (2001). Sweetener preference of C57BL/6ByJ and 129P3/J mice. *Chem Senses* **26**: 905–13.

Bell RL, Kimpel MW, Rodd ZA, Strother WN, Bai F, Peper C L, et al. (2006). Protein expression changes in the nucleus accumbens and amygdala of inbred alcohol-preferring rats given either continuous or scheduled access to ethanol. *Alcohol* **40**: 3–17.

Berger AL, Williams AM, McGinnis MM, Walker BM (2013). Affective cue-induced escalation of alcohol self-administration and increased 22-kHz ultrasonic vocalizations during alcohol withdrawal: role of kappa-opioid receptors. *Neuropsychopharmacol* **38**: 647–54.

Berridge KC, Kringelbach ML (2015). Pleasure systems in the brain. *Neuron* **86**: 646–64.

Biggs TA, Myers RD (1998). Naltrexone and amperozide modify chocolate and saccharin drinking in high alcohol-preferring P rats. *Pharmacol Biochem Behav* **60**: 407–13.

Bodnar RJ (2019). Endogenous opioid modulation of food intake and body weight: Implications for opioid influences upon motivation and addiction. *Peptides* **116**: 42–62.

Carnicella S, Ron D, Barak S (2014). Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. *Alcohol* **48**: 243–52.

Castro DC, Berridge KC (2017). Opioid and orexin hedonic hotspots in rat orbitofrontal cortex and insula. *Proc Natl Acad Sci U S A* **114**: 9125–34.

Chow BL, Sellers EM, Tomkins DM (1997). Effect of naltrexone and its derivatives, nalmefene and naltrindole, on conditioned anticipatory behaviour and saccharin intake in rats. *Behav Pharmacol* **8**: 725–35.

Colantuoni C, Rada P, McCarthy J, Patten C, Avena NM, Chadeayne A, Hoebel BG (2002). Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obes Res* **10**: 478–88.

Colombo G, Maccioni P, Acciaro C, Lobina, C, Loi B, Zaru A, et al. (2014). Binge drinking in alcohol-preferring sP rats at the end of the nocturnal period. *Alcohol* **48**: 301–11.

Cozzoli DK, Courson J, Caruana AL, Miller BW, Greentree DI, Thompson AB, et al. (2012). Nucleus accumbens mGluR5-associated signaling regulates binge alcohol drinking under drinking-in-the-dark procedures. *Alcohol Clin Exp Res* **36**: 1623–33.

Crabbe JC, Metten P, Belknap JK, Spence SE, Cameron AJ, Schlumbohm JP, Huang LC, Barkley-Levenson AM, Ford MM, Phillips TJ (2014). Progress in a replicated selection for elevated blood ethanol concentrations in HDID mice. *Genes Brain Behav* **13**:236–46.

Crabbe JC, Ozburn AR, Metten P, Barkley-Levenson A, Schlumbohm JP, Spence SE, et al. (2017). High Drinking in the Dark (HDID) mice are sensitive to the effects of some clinically relevant drugs to reduce binge-like drinking. *Pharmacol Biochem Behav* **160**: 55–62.

Davidson D, Amit Z (1997). Effect of ethanol drinking and naltrexone on subsequent drinking in rats. *Alcohol* **14**: 581–84.

Davis CA, Levitan RD, Reid C, Carter JC, Kaplan AS, Patte KA, et al. (2009). Dopamine for "wanting" and opioids for "liking": a comparison of obese adults with and without binge eating. *Obesity* **17**: 1220–5.

Dhaher R, McConnell KK, Rodd ZA, McBride WJ, Bell RL (2012). Daily patterns of ethanol drinking in adolescent and adult, male and female, high alcohol drinking (HAD) replicate lines of rats. *Pharmacol Biochem Behav* **102**: 540–8.

Di Lorenzo PM, Kiefer SW, Rice AG, Garcia J (1986). Neural and behavioral responsivity to ethyl alcohol as a tastant. *Alcohol* **3**: 55–61.

Esser MB, Hedden SL, Kanny D, Brewer RD, Gfroerer JC, Naimi TS (2014). Prevalence of alcohol dependence among US adult drinkers, 2009-2011. *Prev Chronic Dis* **11**: 140329.

Fachin-Scheit DJ, Frozino Ribeiro A, Pigatto G, Oliveira Goeldner F, Boerngen de Lacerda R (2006). Development of a mouse model of ethanol addiction: naltrexone efficacy in reducing consumption but not craving. *J Neural Transm* **113**: 1305–21.

Font L, Lujan MA, Pastor R. Involvement of the endogenous opioid system in the psychopharmacological actions of ethanol: the role of acetaldehyde. *Front Behav Neurosci* **7**:93.

Fortuna JL (2010). Sweet preference, sugar addiction and the familial history of alcohol dependence: shared neural pathways and genes. *J Psychoactive Drugs* **42**: 147–51.

Fultz EK, Martin DL, Hudson CN, Kippin TE, Szumlinski KK (2017). Methamphetamine-alcohol interactions in murine models of sequential and simultaneous oral drug-taking. *Drug Alcohol Depend* **177**:178–86.

Gianoulakis C (2001). Influence of the endogenous opioid system on high alcohol consumption and genetic predisposition to alcoholism. *J Psychiatry Neurosci* **26**: 304–18.

Giardino WJ, Ryabinin AE (2013). CRF1 receptor signaling regulates food and fluid intake in the drinking-in-the-dark model of binge alcohol consumption. *Alcohol Clin Exp Res* **37**: 1161–70.

Goings T, Salas-Wright CP, Belgrave FZ, Nelson EJ, Harezlak J, Vaughn MG (2019). Trends in binge drinking and alcohol abstention among adolescents in the US, 2002-2016. *Drug Alcohol Depend* **200**: 115–23.

Gonzales K, Roeber J, KannyD, Tran A, Saiki C, Johnson H, et al. (2014). Alcohol-attributable deaths and years of potential life lost--11 States, 2006-2010. *MMWR Morb Mortal Wkly Rep* **63**: 213–16.

Hayibor LA, Zhang J, Duncan A (2019). Association of binge drinking in adolescence and early adulthood with high blood pressure: findings from the National Longitudinal Study of Adolescent to Adult Health (1994-2008). *J Epidemiol Community Health* **73**: 652–59.

Holgate J, Shariff M, Mu EWH, Bartlett S (2017). A Rat Drinking in the Dark Model for Studying Ethanol and Sucrose Consumption. *Front Behav Neurosci* **11**: 29.

Jeanblanc J, Rolland B, Gierski F, Martinetti MP, Naassila M (2018). Animal models of binge drinking, current challenges to improve face validity. *Neurosci Biobehav Rev* doi: 10.1016/j.neubiorev.2018.05.002.

Jonas DE, Amick HR, Feltner C, Bobashev G, Thomas K, Wines R, et al. (2014). Pharmacotherapy for adults with alcohol use disorders in outpatient settings: a systematic review and meta-analysis. *JAMA* **311**:1889-900.

June HL, Cummings R, Eiler WJA, Foster KL, McKay PF, Seyoum R, et al. (2004). Central opioid receptors differentially regulate the nalmefene-induced suppression of ethanol- and saccharin-reinforced behaviors in alcohol-preferring (P) rats. *Neuropsychopharmacology* **29**: 285–99.

Kamdar NK, Miller SA, Syed YM, Bhayana R, Gupta T, Rhodes JS (2007). Acute effects of naltrexone and GBR 12909 on ethanol drinking-in-the-dark in C57BL/6J mice. *Psychopharmacol* **192**: 207–17.

Kamens HM, SilvaC, PeckC, Miller CN (2018). Varenicline modulates ethanol and saccharin consumption in adolescent male and female C57BL/6J mice. *Brain Res Bull* **138**: 20–5.

Kampov-Polevoy AB, Garbutt JC, Janowsky DS (1999). Association between preference for sweets and excessive alcohol intake: a review of animal and human studies. *Alcohol Alcohol* **34**: 386–95.

Kampov-Polevoy AB, Tsoi MV, Zvartau EE, Neznanov NG, Khalitov E (2001). Sweet liking and family history of alcoholism in hospitalized alcoholic and non-alcoholic patients. *Alcohol Alcohol* **36**: 165–70.

Kampov-Polevoy AB, Ziedonis D, Steinberg ML, Pinsky I, Krejci J, Eick C, et al. (2003). Association between sweet preference and paternal history of alcoholism in psychiatric and substance abuse patients. *Alcohol Clin Exp Res* **27**: 1929–36.

Kanny D, Naimi TS, Liu Y, Lu H, Brewer RD (2018). Annual Total Binge Drinks Consumed by U.S. Adults, 2015. *Am J Prevent Med* **54**: 486–96.

Kelley AE, Bakshi VP, Haber SN, Steininger TL, Will MJ, Zhang M (2002). Opioid modulation of taste hedonics within the ventral striatum. *Physiol Behav* **76**: 365–77.

Kiefer F, Jahn H, Tarnaske T, Helwig H, Briken P, Holzbach R, et al. (2003). Comparing and combining naltrexone and acamprosate in relapse prevention of alcoholism: a double-blind, placebo-controlled study. *Arch Gen Psychiatry* **60**: 92–99.

Le AD, Poulos CX, Quan B, Chow S (1993). The effects of selective blockade of delta and mu opiate receptors on ethanol consumption by C57BL/6 mice in a restricted access paradigm. *Brain Res* **630**: 330–2.

Leggio L, Addolorato G, Cippitelli A, Jerlhag E, Kampov-Polevoy AB, Swift RM (2011). Role of feeding-related pathways in alcohol dependence: A focus on sweet preference, NPY, and ghrelin. *Alcohol Clin Exp Res* **35**: 194–202.

Lowery EG, Spanos M, Navarro M, Lyons AM, Hodge CW, Thiele TE (2010). CRF-1 antagonist and CRF-2 agonist decrease binge-like ethanol drinking in C57BL/6J mice independent of the HPA axis. *Neuropsychopharmacol* **35**: 1241–52.

Lynch WC, Libby L (1983). Naloxone suppresses intake of highly preferred saccharin solutions in food deprived and sated rats. *Life Sci* **33**: 1909–14.

Lyons AM, Lowery EG, Sparta DR, Thiele TE (2008). Effects of food availability and administration of orexigenic and anorectic agents on elevated ethanol drinking associated with drinking in the dark procedures. *Alcohol Clin Exp Res* **32**: 1962–8.

Melendez RI (2011). Intermittent (every-other-day) drinking induces rapid escalation of ethanol intake and preference in adolescent and adult C57BL/6J mice. *Alcohol Clin Exp Res* **35**: 652–8.

Morales I, Currie PJ, Hackenberg TD, Pastor R (2017). Opioidergic and dopaminergic modulation of cost/benefit decision-making in Long Evans Rats. *Physiol Behav* **179**: 442–50.

Middaugh LD, Szumlinski KK, Van Patten Y, Marlowe ALB, Kalivas PW (2003). Chronic ethanol consumption by C57BL/6 mice promotes tolerance to its interoceptive cues and increases extracellular dopamine, an effect blocked by naltrexone. *Alcohol Clin Exp Res* **27**: 1892–900.

National Institute on Alcohol Abuse and Alcoholism (2004) *NIAAA Council Approves Definition of Binge Drinking*.

Navarro M, Luhn KL, Kampov-Polevoy AB, Garbutt JC, Thiele TE (2019). Bupropion, Alone and in Combination with Naltrexone, Blunts Binge-Like Ethanol Drinking and Intake Following Chronic Intermittent Access to Ethanol in Male C57BL/6J Mice. *Alcohol Clin Exp Res* **43**: 783–90.

Olszewski PK, Levine AS (2007). Central opioids and consumption of sweet tastants: when reward outweighs homeostasis. *Physiol Behav* **91**: 506-12.

Olszewski PK, Alsio J, Schioth HB, Levine AS (2011). Opioids as facilitators of feeding: can any food be rewarding? *Physiol Behav* **104**: 105–10.

Olszewski PK, Wood EL, Klockars A, Levine AS (2019). Excessive Consumption of Sugar: an Insatiable Drive for Reward. *Curr Nutr Rep* **8**: 120–8.

Pastor R, Aragon CM (2006). The role of opioid receptor subtypes in the development of behavioral sensitization to ethanol. *Neuropsychopharmacol* **31**: 1489–99.

Pastor R, Sanchis-Segura C, Aragon CM (2005). Effect of selective antagonism of mu(1)-, mu(1/2)-, mu(3)-, and delta-opioid receptors on the locomotor-stimulating actions of ethanol. *Drug Alcohol Dep* **78**: 289–95.

Pastor R, Kamens HM, McKinnon CS, Ford MM, Phillips TJ (2010). Repeated ethanol administration modifies the temporal structure of sucrose intake patterns in mice: effects associated with behavioral sensitization. *Addict Biol* **15**: 324–335.

Pastor R, Font L, Miquel M, Phillips TJ, Aragon CM (2011). Involvement of the beta-endorphin neurons of the hypothalamic arcuate nucleus in ethanol-induced place preference conditioning in mice. *Alcohol Clin Exp Res* **35**: 2019–29.

Patrick ME, Schulenberg JE (2013). Prevalence and predictors of adolescent alcohol use and binge drinking in the United States. *Alcohol Res* **35**: 193–200.

Phillips TJ, Wenger CD, Dorow JD (1997). Naltrexone effects on ethanol drinking acquisition and on established ethanol consumption in C57BL/6J mice. *Alcohol Clin Exp Res* **21**: 691–702.

Pietraszek A, Gregersen S, Hermansen K (2010). Alcohol and type 2 diabetes. A review. *Nutr Metab Cardiovasc Dis* **20**: 366–75.

Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet* **373**: 2223–33.

Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiol Behav* **84**: 53–63.

Rhodes JS, Ford MM, Yu CH, Brown LL, Finn DA, Garland T Jr, Crabbe JC (2007). Mouse inbred strain differences in ethanol drinking to intoxication. *Genes Brain Behav* **6**:1-18.

Ripley TL, Sanchez-Roige S, Bullmore ET, Mugnaini M, Maltby K, Miller SR, et al. (2015). The novel mu-opioid antagonist, GSK1521498, reduces ethanol consumption in C57BL/6J mice. *Psychopharmacol* **232**: 3431–41.

Rodd ZA, Bell RL, Kuc KA, Murphy JM, Lumeng L, McBride WJ (2009). Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of high-alcohol-drinking (HAD) rats. *Addict Biol* **14**: 152–64.

Rosoff DB, Charlet K, JungJ, Lee J, Muench C, Luo A, et al. (2019). Association of High-Intensity Binge Drinking With Lipid and Liver Function Enzyme Levels. *JAMA Netw Open* **2**: 195844.

Sacks JJ, Gonzales KR, Bouchery EE, Tomedi LE, Brewer RD (2015). 2010 National and State Costs of Excessive Alcohol Consumption. *Am J Prev Med* **49**: 73–9.

Schulte E, Avena NM, Gearhardt AN (2015). Which foods may be addictive? The roles of processing, fat content, and glycemic load. *PloS One* **10**: 0117959.

Schulte EM, Yokum S, Potenza MN, Gearhardt AN (2016). Neural systems implicated in obesity as an addictive disorder: from biological to behavioral mechanisms. *Prog Brain Res* **223**: 329–46.

Scinska A, Koros E, Habrat B, Kukwa A, Kostowski W, Bienkowski P (2000). Bitter and sweet components of ethanol taste in humans. *Drug Alcohol Dep* **60**: 199–206.

Serra S, Brunetti G, Vacca G, Lobina C, Carai MAM, Gessa GL, Colombo G (2003). Stable preference for high ethanol concentrations after ethanol deprivation in Sardinian alcohol-preferring (sP) rats. *Alcohol* **29**: 101–8.

Shabani S, Houlton SK, Hellmuth L, Mojica E, Mootz JRK, Zhu Z, et al. (2016). A Mouse Model for Binge-Level Methamphetamine Use. *Front Neurosci* **10**: 493.

Sharpe AL, Samson HH (2001). Effect of naloxone on appetitive and consummatory phases of ethanol self-administration. *Alcohol Clin Exp Res* **25**: 1006–11.

Spanagel R, Holter SM, Allingham K, Landgraf R, Zieglgansberger W (1996). Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat. *Eur J Pharmacol* **305**: 39–44.

Sprow GM, Thiele TE (2012). The neurobiology of binge-like ethanol drinking: evidence from rodent models. *Physiol Behav* **106**: 325–31.

Stahre M, RoeberJ, Kanny D, Brewer RD, Zhang X (2014). Contribution of excessive alcohol consumption to deaths and years of potential life lost in the United States. *Prev Chronic Dis* **11**: E109.

Sundell L, Salomaa V, Vartiainen E, Poikolainen K, Laatikainen T (2008). Increased stroke risk is related to a binge-drinking habit. *Stroke* **39**: 3179–84.

Szumlinski KK, Coelho MA, Tran T, Stailey N, Lieberman D, Gabriella I, et al. (2019). Who is HOT and who is LOT? Detailed characterization of prescription opioid-induced changes in behavior between 129P3/J and 129S1/SvlmJ mouse substrains. *Genes Brain Behav* **e12609**. doi: 10.1111/gbb.12609.

Tabarin A, Diz-Chaves Y, Consoli D, Monsaingeon M, Bale TL, Culler MD, et al. (2007). Role of the corticotropin-releasing factor receptor type 2 in the control of food intake in mice: a meal pattern analysis. *Eur J Neurosci* **26**: 2303–14.

Tarragón E, Balino P, Aragon CM, Pastor R (2012). Ethanol drinking-in-the-dark facilitates behavioral sensitization to ethanol in C57BL/6J, BALB/cByJ, but not in mu-opioid receptor deficient CXBK mice. *Pharmacol Biochem Behav* **101**: 14–23.

Tavolacci MP, Berthon Q, Cerasuolo D, Dechelotte, Ladner J, Baguet A (2019). Does binge drinking between the age of 18 and 25 years predict alcohol dependence in adulthood? A retrospective case-control study in France. *BMJ Open* ***9****: e026375.

Thiele TE, Navarro M (2014) "Drinking in the dark" (DID) procedures: a model of binge-like ethanol drinking in non-dependent mice. *Alcohol* **48**: 235–41.

Tordoff MG, Bachmanov AA (2003a). Influence of the number of alcohol and water bottles on murine alcohol intake. *Alcohol Clin Exp Res* **27**: 600–6.

Tordoff MG, Bachmanov AA (2003b). Mouse taste preference tests: why only two bottles? *Chem Senses* **28**: 315–24.

Treasure J, Eid L (2019). Eating disorder animal model. *Curr Opin Psychiatry* doi: 10.1097/YCO.0000000000000550.

Volkow ND, Wang GJ, Tomasi D, Baler RD. (2013). Obesity and addiction: neurobiological overlaps. *Obes Rev* **14**: 2–18.

Volkow ND, Wise RA, Baler R (2017). The dopamine motive system: implications for drug and food addiction. *Nat Rev Neurosci* **18**: 741–52.

Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP (1992). Naltrexone in the treatment of alcohol dependence. *Archi Gen Psychiatry* **49**: 876–80.

Wilcox MV, Cuzon Carlson VC, Sherazee N, Sprow GM, Bock R, Thiele TE, et al. (2014). Repeated binge-like ethanol drinking alters ethanol drinking patterns and depresses striatal GABAergic transmission. *Neuropsychopharmacol* **39**: 579–94.

Wiss DA, Avena N, Rada P (2018). Sugar Addiction: From Evolution to Revolution. *Front Psychiatry* **9**: 545.

Zhang M, Kelley AE (2002). Intake of saccharin, salt, and ethanol solutions is increased by infusion of a mu opioid agonist into the nucleus accumbens. *Psychopharmacol* **159**: 415–23.

Zhou Y, Rubinstein M, Low MJ, Kreek MJ (2017a). Hypothalamic-specific proopiome-lanocortin deficiency reduces alcohol drinking in male and female mice. *Genes Brain Behav* **16**: 449–61.

Zhou Y, Crowley RS, Ben K, Prisinzano TE, Kreek MJ (2017b). Synergistic blockade of alcohol escalation drinking in mice by a combination of novel kappa opioid receptor agonist Mesyl Salvinorin B and naltrexone. *Brain Res* **1662**: 75–86.

Zhou Y, Leri F, Low MJ, Kreek MJ (2019). Sex differences in the effect of bupropion and naltrexone combination on alcohol drinking in mice. *Pharmacol Biochem Behav* **181**: 28–36.

Table 1. Effects of naltrexone (NTX) on EtOH intake (mL ± SEM)

		NTX Dose (mg/kg)			
	Time (h)	**0**	**4**	**8**	**16**
Total Intake	**0-2**	0.74 ± 0.07	0.53 ± 0.05	0.44 ± 0.05	0.37 ± 0.07
	2-4	0.57 ± 0.05	0.55 ± 0.06	0.45 ± 0.06	0.33 ± 0.05
	0-4	1.31 ± 0.11	1.08 ± 0.10	0.89 ± 0.06*	0.70 ± 0.12**
Time (h)	**Concentration**				
0-2	**5%**	0.10 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02
	10%	0.13 ± 0.03	0.09 ± 0.03	0.11 ± 0.02	0.08 ± 0.04
	20%	0.28 ± 0.04	0.17 ± 0.03	0.15 ± 0.03*	0.09 ± 0.02**
	40%	0.23 ± 0.02	0.21 ± 0.02	0.12 ± 0.02	0.13 ± 0.03
2-4	**5%**	0.07 ± 0.02	0.08 ± 0.02	0.09 ± 0.03	0.06 ± 0.03
	10%	0.10 ± 0.03	0.08 ± 0.02	0.15 ± 0.04	0.07 ± 0.02
	20%	0.25 ± 0.03	0.23 ± 0.04	0.14 ± 0.01	0.07 ± 0.03**
	40%	0.15 ± 0.01	0.16 ± 0.02	0.07 ± 0.02	0.13 ± 0.04
0-4	**5%**	0.17 ± 0.03	0.14 ± 0.04	0.15 ± 0.03	0.13 ± 0.04
	10%	0.23 ± 0.04	0.17 ± 0.04	0.26 ± 0.05	0.15 ± 0.04
	20%	0.53 ± 0.06	0.40 ± 0.06	0.29 ± 0.03**	0.16 ± 0.03**
	40%	0.38 ± 0.02	0.37 ± 0.03	0.19 ± 0.03*	0.26 ± 0.07

Top panel shows the effects of NTX (0, 4, 8 or 16 mg/kg) on total EtOH intake (mL) collapsed across different concentrations for the 0-2 h, 2-4 h, and 0-4 h time periods; *$p < 0.05$ different from 0 mg/kg, **$p < 0.01$ different from 0 and 4 mg/kg. Bottom panels show the effects of NTX on EtOH drunk from each individual tube (concentration); *$p < 0.05$, **$p < 0.01$ relative to 0 mg/kg for each respective time period and concentration. For the 20% EtOH, 16 mg/kg NTX groups **$p < 0.01$ also indicates different from 4 mg/kg (2-4 h and 0-4 h time periods).

Table 2. Effects of naltrexone (NTX) on sucrose intake (mL ± SEM)

		NTX Dose (mg/kg)			
	Time (h)	0	4	8	16
Total Intake	0-2	1.29 ± 0.08	1.15 ± 0.06	0.95 ± 0.09*	0.64 ± 0.09**
	2-4	0.86 ± 0.09	0.80 ± 0.04	0.75 ± 0.06	0.87 ± 0.09
	0-4	2.15 ± 0.09	1.95 ± 0.07	1.70 ± 0.10*	1.51 ± 0.16**
Time (h)	**Concentration**				
0-2	5%	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	10%	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.03	0.02 ± 0.01
	20%	0.07 ± 0.02	0.05 ± 0.03	0.05 ± 0.03	0.03 ± 0.03
	40%	1.14 ± 0.08	1.03 ± 0.07	0.83 ± 0.07**	0.57 ± 0.08**
2-4	5%	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.05 ± 0.03
	10%	0.04 ± 0.03	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	20%	0.02 ± 0.01	0.03 ± 0.03	0.05 ± 0.02	0.04 ± 0.02
	40%	0.78 ± 0.06	0.75 ± 0.05	0.65 ± 0.08	0.77 ± 0.11
0-4	5%	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.07 ± 0.03
	10%	0.07 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	0.03 ± 0.01
	20%	0.09 ± 0.02	0.08 ± 0.05	0.10 ± 0.05	0.07 ± 0.05
	40%	1.92 ± 0.09	1.78 ± 0.09	1.48 ± 0.12**	1.34 ± 0.17**

Top panel indicates effects of NTX on overall sucrose consumption (mL) during the three time periods observed; *$p < 0.05$ and **$p < 0.01$ relative to 0 mg/kg. The NTX 16 mg/kg group (0-4 h) was also different ($p < 0.05$) from NTX 4 mg/kg for the same time period. Concentration-specific effects of NTX are show on lower panels; **$p < 0.01$ indicates differences compared to NTX 0. For 40% sucrose, NTX 8 and 16 mg/kg groups were also different ($p < 0.05$) from NTX 4 mg/kg during the 0-2 h and 0-4 h time periods.

Table 3. Effects of naltrexone (NTX) on saccharine intake (mL ± SEM)

		NTX Dose (mg/kg)			
	Time (h)	**0**	**4**	**8**	**16**
Total Intake	**0-2**	1.59 ± 0.11	1.35 ± 0.11	1.27 ± 0.07	1.15 ± 0.17*
	2-4	1.13 ± 0.11	1.19 ± 0.13	1.24 ± 0.12	1.39 ± 0.13
	0-4	2.72 ± 0.18	2.54 ± 0.20	2.51 ± 0.15	2.54 ± 0.27
Time (h)	**Concentration**				
0-2	**0.13%**	0.20 ± 0.05	0.15 ± 0.04	0.14 ± 0.04	0.11 ± 0.02
	0.26%	0.24 ± 0.04	0.24 ± 0.08	0.24 ± 0.04	0.21 ± 0.05
	0.53%	0.37 ± 0.07	0.35 ± 0.06	0.36 ± 0.06	0.51 ± 0.17
	1.06%	0.78 ± 0.11	0.61 ± 0.08	0.53 ± 0.08	0.32 ± 0.07**
2-4	**0.13%**	0.18 ± 0.04	0.16 ± 0.07	0.12 ± 0.03	0.12 ± 0.04
	0.26%	0.15 ± 0.04	0.22 ± 0.06	0.18 ± 0.04	0.23 ± 0.06
	0.53%	0.27 ± 0.04	0.33 ± 0.07	0.41 ± 0.08	0.63 ± 0.14**
	1.06%	0.53 ± 0.07	0.48 ± 0.07	0.53 ± 0.07	0.41 ± 0.08
0-4	**0.13%**	0.38 ± 0.08	0.31 ± 0.08	0.26 ± 0.07	0.23 ± 0.05
	0.26%	0.39 ± 0.07	0.46 ± 0.14	0.42 ± 0.07	0.44 ± 0.10
	0.53%	0.64 ± 0.10	0.68 ± 0.12	0.77 ± 0.12	1.14 ± 0.29
	1.06%	1.31 ± 0.15	1.09 ± 0.11	1.06 ± 0.13	0.73 ± 0.14**

Top section indicates total volume of saccharine drank after each NTX (0, 4, 8 or 16 mg/kg) dose. NTX 16 mg/kg decreased saccharine drinking (*$p < 0.05$ relative to NTX 0 mg/kg) during the first 2-h block. When individual concentrations were considered, the highest dose of NTX decreased intake of 1.06% and increase 0.53% saccharine solutions (**$p < 0.01$ relative to 0 mg/kg for each respective time period).

Table 4. Effects of naltrexone (NTX) on sucrose, saccharin and ethanol (EtOH) intake (mL ± SEM)

	Time (h)	NTX Dose (mg/kg)			
		0	**4**	**8**	**16**
Total Intake	**0-2**	2.34 ± 0.19	1.80 ± 0.18	1.84 ± 0.17	1.96 ± 0.10
	2-4	2.57 ± 0.16	2.00 ± 0.18	1.74 ± 0.11	2.02 ± 0.09
	0-4	4.91 ± 0.30	3.80 ± 0.32**	3.58 ± 0.26**	3.98 ± 0.18*
Time (h)	**Tastant**				
0-2	**Saccharin 0.13%**	1.41 ± 0.13	1.12 ± 0.14	1.23 ± 0.13	1.24 ± 0.10
	Sucrose 5%	0.81 ± 0.12	0.61 ± 0.10	0.46 ± 0.08	0.63 ± 0.09
	EtOH 20%	0.12 ± 0.07	0.07 ± 0.02	0.15 ± 0.07	0.09 ± 0.05
2-4	**Saccharin 0.13%**	1.57 ± 0.10	1.28 ± 0.12	1.17 ± 0.09	1.31 ± 0.10
	Sucrose 5%	0.92 ± 0.12	0.67 ± 0.10	0.47 ± 0.05	0.60 ± 0.08
	EtOH 20%	0.08 ± 0.03	0.05 ± 0.02	0.10 ± 0.07	0.11 ± 0.07
0-4	**Saccharin 0.13%**	2.98 ± 0.20	2.40 ± 0.22	2.40 ± 0.20	2.55 ± 0.17
	Sucrose 5%	1.73 ± 0.22	1.28 ± 0.19	0.93 ± 0.12	1.23 ± 0.15
	EtOH 20%	0.20 ± 0.07	0.12 ± 0.02	0.25 ± 0.10	0.20 ± 0.08

Table 4 shows total saccharin, sucrose and EtOH drinking (simultaneously available) after NTX treatment (0, 4, 8 or 16 mg/kg). Top panel; all NTX doses (4, 8, 16 mg/kg) decreased total solution consumption during the 4-h test period; *$p < 0.05$ and **$p < 0.01$ relative to NTX 0 mg/ kg. Bottom panels show individual tastant consumptions as a function of time and NTX dose.

Figure Legends

Figure 1. EtOH intake (g/kg) as a function of EtOH concentration availability.
Intakes obtained with separate groups (n=12-15) with access (4 h) to one, three or four
EtOH tubes. From left to right, bars (mean ± SEM) represent access to: A, one tube with
20% EtOH; B, one tube with 40% EtOH; C, three simultaneously available tubes with
5%, 10% and 20% EtOH; D, three simultaneously available tubes with 20%, 30% and
40% EtOH; E, four simultaneously available tubes with 5%, 10%, 20% and 40% EtOH.
*$p < 0.05$, **$p < 0.01$ indicates different from A. Group E was also found to be different
from B ($p < 0.05$).

Figure 2. Effects of naltrexone (NTX) on EtOH intake (g/kg). Panel A shows the
effect of NTX (0, 4, 8 or 16 mg/kg) on total EtOH intake (collapsed on concentration)
during 0-2 h, 2-4 h and 0-4 h time periods. Panels B, C and D show NTX effects on
EtOH intake as a function of concentration for the two 2-h periods and total 0-4 h
period, respectively. *$p < 0.05$, **$p < 0.01$ indicates different from NTX 0 mg/kg at the
same EtOH concentration. Bars represent mean ± SEM; n = 15 per group.

Figure 3. Effects of naltrexone (NTX) on sucrose intake (g/kg). Effects of NTX for
total intakes during 0-2 h, 2-4 h and 0-4 h time periods, combining all sucrose
concentrations, are shown on panel A; *$p < 0.05$, different from NTX 0 mg/kg for each
respective time period. Panels B, C and D show NTX effects on sucrose intake as a
function of concentration during the two 2-h periods and total 0-4 h period, respectively.
**$p < 0.01$ indicates different from NTX 0 mg/kg at the same EtOH concentration. Bars
represent mean ± SEM; n = 15 per group.

Figure 4. Effects of naltrexone (NTX) on saccharin intake (g/kg). Panel A shows the
effect of NTX on total intake (combining all saccharin concentrations) during the 0-2 h,
2-4 h and 0-4 h time periods; *$p < 0.05$, different from NTX 0 mg/kg for each
respective time period. Panels B, C and D show NTX effects on saccharin intake at
different concentrations and the effects of NTX during the two 2-h periods and total 0-4
h period, respectively. *$p < 0.05$, **$p < 0.01$ indicates different from NTX 0 mg/kg at
the same EtOH concentration. Bars represent mean ± SEM; n = 16 per group.

**Figure 5. Effects of naltrexone (NTX) on simultaneously available saccharin (S),
sucrose (SU) and EtOH (E) intake (ml/g).** Effects of NTX on total intakes during 0-2
h, 2-4 h and 0-4 h time periods, combining ml drank from saccharin, sucrose and EtOH
tubes are shown on panel A; *$p < 0.05$, **$p < 0.01$ different from NTX 0 mg/kg. Panels
B, C and D show NTX effects on the three different drinking solutions during the two 2-
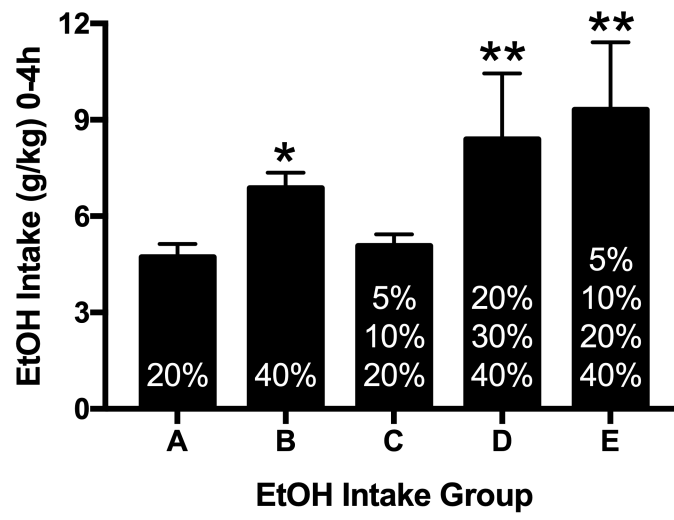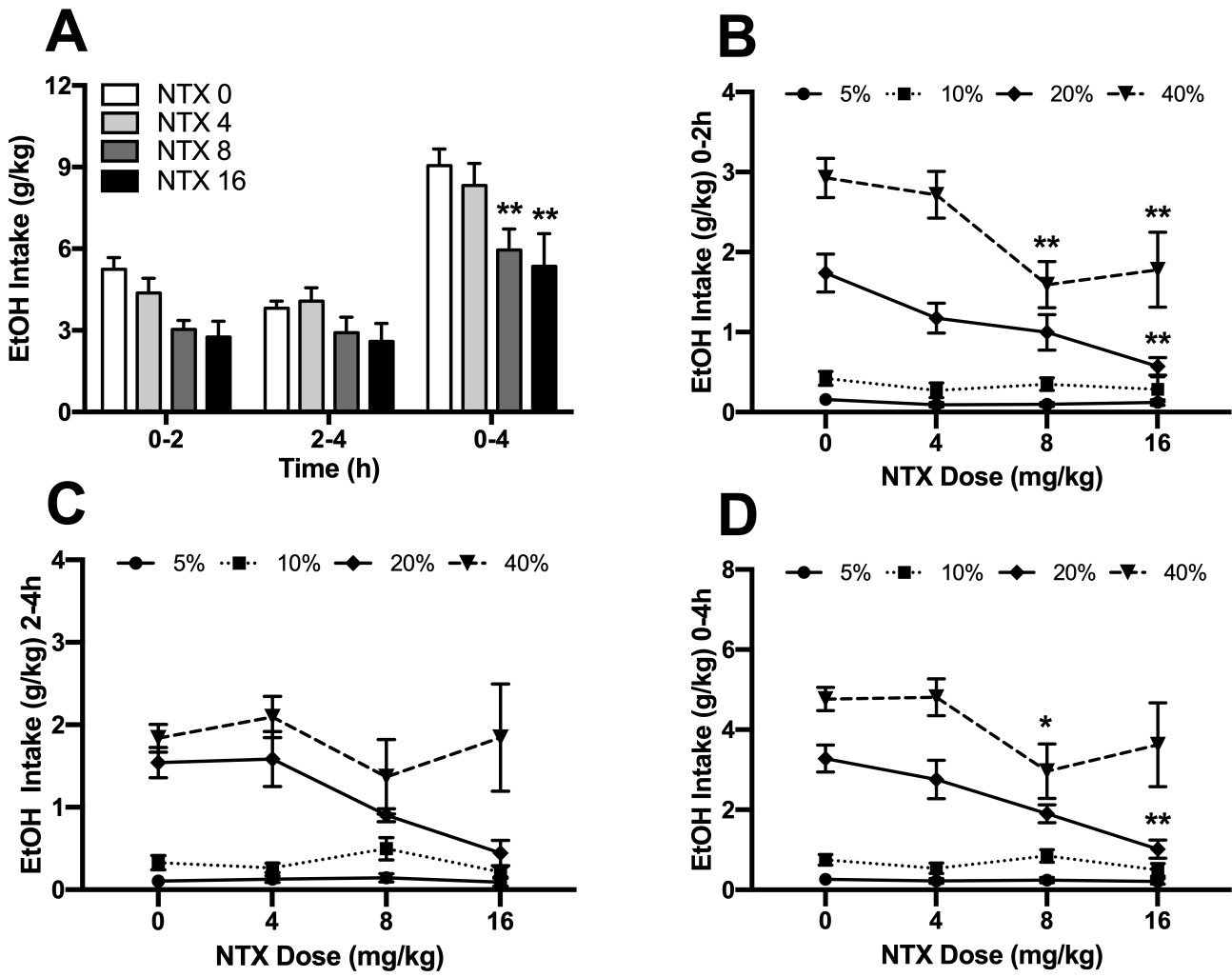h periods and total 0-4 h period, respectively. Bars represent mean ± SEM; n = 15 per
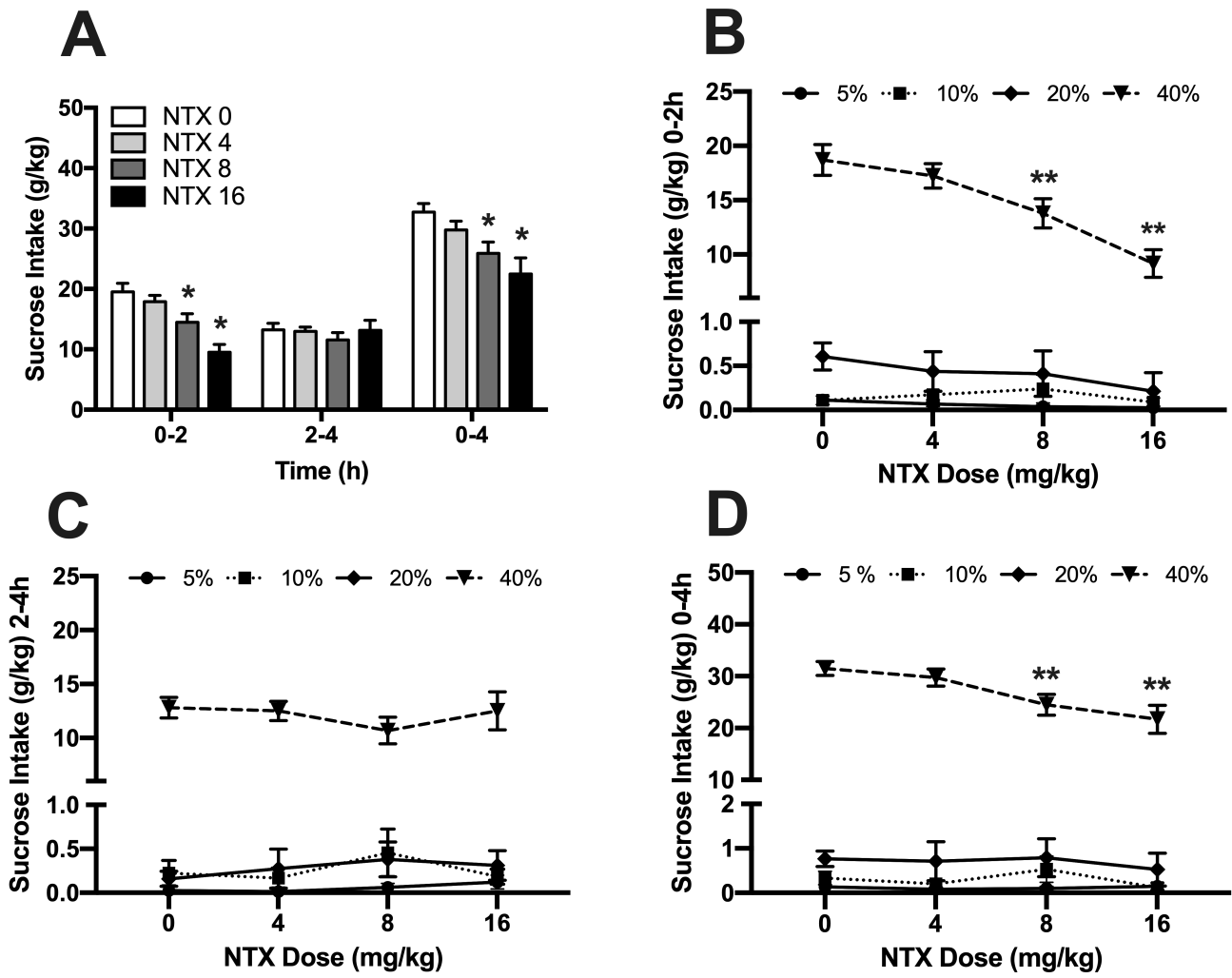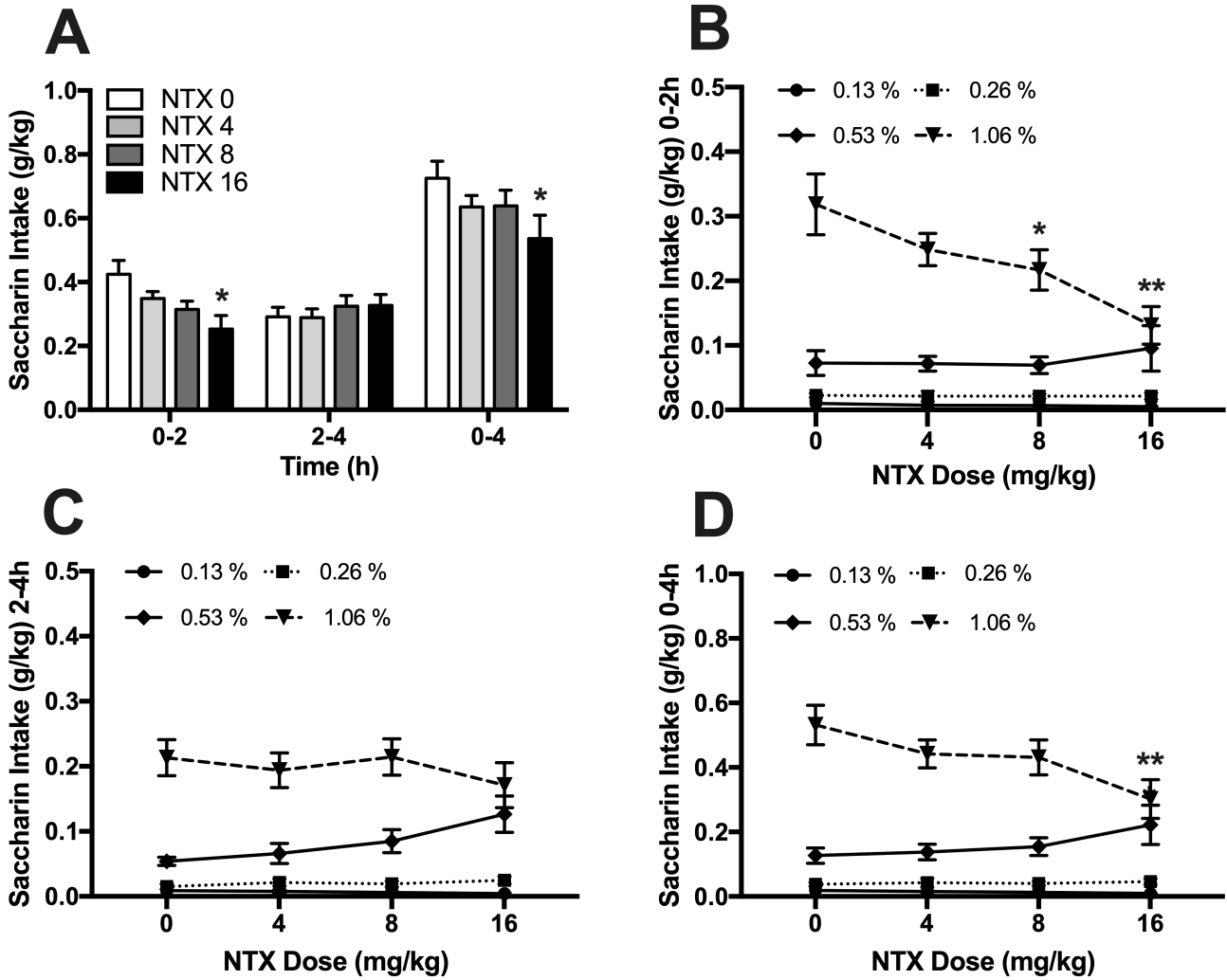group.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5