Binge eating promotes ethanol self-administration in female rats with a history of intermittent ethanol exposure at adolescence

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Abstract. *Background:* Ethanol drinking begins during adolescence and, particularly when occurs in a binge-like pattern, exerts lingering adverse consequences. Pre-clinical studies indicate that intermittent ethanol exposure (IEA, a model of repeated ethanol intoxication), or binge eating (BE) can increase subsequent ethanol consumption. It is unknown if the promoting effects of BE upon ethanol drinking are found in female rats and are modulated by IEA at adolescence. This study assessed interactive effects between IEA and BE, upon ethanol drinking. *Methods:* Female Wistar rats were given 4.0 g/kg ethanol, every other day from postnatal day 25 to 45. At adulthood, they were exposed to sessions in which a brief offering of a sizeable portion of highly palatable sugary pills was followed by a 120-min exposure to an ethanol bottle. *Results:* Exploratory activity and recognition memory was not affected by the IEA. Glutathione peroxidase and catalase activity, and lipid peroxidation (measured in blood and brain at the end of the procedure) were not significantly affected by IEA or BE exposure. BE alone had a mild promoting effect on ethanol ingestion. Those rats that underwent IEA and BE, however, exhibited heightened and sustained ethanol self-administration (average of 2.12 g/kg/120 min, vs 1.15 g/kg/120 min of the other groups), that persisted throughout the BE sessions. IEA and a history of BE also promoted ethanol intake or preference in a two-bottle endpoint test. *Conclusion:* The study suggests that exposure to IEA exerts, when followed by BE at adulthood, promoting effects upon ethanol intake, particularly at concentrations ≥6%.

Keywords: intermittent ethanol exposure, binge drinking, binge eating, Wistar rats, females

1. Introduction

Alcohol (ethanol) intake usually begins and escalates at adolescence and is associated with a significant array of immediate negative consequences, such as greater possibility of interpersonal violence (Bravo et al., 2019). The early contact with ethanol is also associated with greater likelihood of exhibiting ethanol use or alcohol-use disorders at adulthood (Guttmannova et al., 2012). The adolescent ethanol exposure may alter the normal developmental trajectories of transmitter systems that process ethanol (Pascual et al., 2009), or make the individual more vulnerable to the promoting effect that other factors (Füllgrabe et al., 2007) exert on ethanol intake at adulthood.

We have reported (Ruiz-Leyva et al., 2022) that the rapid ingestion of hyperpalatable food heightens, in male Wistar rats, binge ethanol drinking. In this preparation the rats are daily and briefly (3 min) exposed to a large quantity of sugary pellets. The rats display progressive acceptance of the palatable food, eating most of it (in the allotted brief time, thus showing binge eating, BE) after five sessions. This engagement in BE was associated with heightened ethanol self- administration, as measured in a limited access procedure immediately after the ingestion of the food. These results (Ruiz-Leyva et al., 2022) pinpoint to interactions between BE and ethanol drinking, that can favor the development of ethanol use problems.

These findings are consistent with studies indicating substantial comorbidity between eating disorders and substance use disorders (Root et al., 2010), and with suggestions that common neurobiological mechanisms underly both phenomena (Lindgren et al., 2018). Notably, a positive correlation has been shown between the ingestion of sweet solutions and the ingestion of ethanol (Blizard and McClearn, 2000); and sucrose intake is significantly

greater in rats selectively bred for high ethanol preference, relative to rats bred for low ethanol preference (Stewart et al., 1994).

Ethanol exposure at adolescence is modelled by giving rats or mice administrations of ethanol in an intermittent and binge-like fashion (i.e., doses between 2.5 to 4 g/kg/d) across postnatal days (PD) 25-42, approximately. Intermittent ethanol administration (IEA) at adolescence alters glutamatergic and dopaminergic transmission (Trantham-Davidson et al., 2017) and the neural pruning of these systems (Pascual et al., 2009). IEA can also increase subsequent ethanol consumption (Fabio et al., 2014), albeit the results concerning this outcome are mixed, with several studies failing to report alterations in ethanol intake at adulthood (Towner and Varlinskaya, 2020). Also uncertain is whether the promoting effects of BE upon ethanol drinking, as shown in Ruiz-Leyva et al. (2022), are found in females and are modulated by IEA at adolescence. The present study filled this void by exposing female Wistar rats to an IEA procedure at adolescence; and then assessed ethanol self-administration via an adapted version of the "binge eating to binge drinking" model (Ruiz-Leyva et al., 2022).

Specifically, the rats were given 4.0 g/kg ethanol, every other day from PD25 to 45. At adulthood they were exposed to repeated sessions in which a 120-min exposure to an ethanol bottle was immediately preceded by a brief (i.e., 3 min) offering of a sizeable portion of highly palatable sugary pills. The effects of IEA on exploratory activity and recognition memory were also tested. Repeated ethanol exposure, particularly in a binge-like pattern, promotes oxidative damage, via the production of reactive oxygen species (Ojeda et al., 2022). Thus, after finding that IEA and BE had synergic effects, heightening ethanol self-administration, we challenged the rats with a binge-like administration of ethanol and tested

their antioxidant capacities, by measuring Glutathione Peroxidase (GPx) activity, Catalase (CAT) activity, and lipid peroxidation status in blood and brain.

2. Materials and Methods

2.1 Experimental design

We employed a 2 (Ethanol exposure at adolescence: IEA *vs*. Control group administered vehicle) × 2 (Control eating condition or BE [i.e., 1 or 12 sugar pills, respectively]) factorial design, with 9-12 rats in each cell of the design.

2.2 Subjects

We employed 41 female rats, derived from 12 litters born and reared at INIMEC-CONICET-UNC (Córdoba, Argentina). Litter effects were controlled by not including more than one female from each litter in each cell of the design. The rats were maintained under a 12 h/12 h light/dark cycle at 22-24ºC, and kept in pairs since weaning and until PD75, when they were housed individually. The procedures endorsed the Declaration of Helsinki, the Guide for the Care and Use of Laboratory Animals as adopted by the NIH/EU and were approved by the Institutional Animal Care and Use Committee at INIMEC-CONICET-UNC.

2.3 Procedures (see timeline in Figure 1)

IEA at adolescence (PDs 25-45)

Similar to Dannenhoffer et al. (2021), the rats were administered every other day with ethanol (4.0 g/kg, intragastrical, concentration: 25% v/v, Porta Hnos, Córdoba, Argentina) or an equivalent volume of tap water, between PDs 25 and 45 (for a total of 11 administrations,

see Figure 1), thus spanning the adolescent developmental stage (Burke and Miczek, 2014; Doremus-Fitzwater and Spear, 2016). The administration involved the introduction of polyethylene tubing, connected to a 10 ml syringe, into the oral cavity, which was then guided to the stomach.

FIGURE 1 HERE

Open Field (OF, PD 50) and Novel object recognition test (NOR, PDs 51-52).

On PD50 (see Figure 1) all rats were placed in the OF arena (a rectangle made of Plexiglas, 50cm × 50cm × 50cm), for 10 min. Distance travelled (cm) was measured, via an automated system. Twenty-four hours later we tested, in a sub-set of rats (10 females exposed to ethanol and 10 exposed to vehicle) recognition memory (Salguero et al., 2020).

During the familiarization phase the rats were placed in the OF arena for 5 min, which featured two identical objects (i.e., A and A', opaque glass flasks) in the upper corners. A 5-min test session took place 24 h later, during which one of the objects was replaced by a taller and slightly clearly colored object (B). The testing phase was recorded and analyzed for time spent near the objects. Time spent in proximity to object B was taken as an indicator of short-term memory (Vogel-Ciernia and Wood, 2014). We calculated a relative discrimination index (Di) by subtracting time spent exploring the familiar object from time spent exploring the novel object. The outcome was divided by the total time spent exploring both objects.

Binge eating and Ethanol Intake (i.e., binge drinking and two-bottle choice) procedures (PDs 80-96, see Figure 1)

We adapted a procedure (Ruiz-Leyva et al., 2022), developed to induce the ingestion of a relatively high amount of carbohydrate-based food in a short timeframe. Specifically,

the rats were given 1 (controls rats) or 12 highly palatable sugary pills to eat in 3 minutes (1.16 or 13.86 kcal, respectively), daily for 10 days, beginning at PD80. The rationale for given control rats a single sugary pill (rather than 12 pellets of a standard diet) is that the procedure aims at exposing the rats to carbohydrate-based food in a manner that triggers BE. Thus, a proper control for this objective implies rats exposed to the same type of food yet in manner that does not yield BE. Our preliminary data indicated that it was possible for the experimental rats to eat the whole ration in 3 minutes, provided the ingestion was swiftly and continuous. As reported in Ruiz-Leyva et al. (2022) the ingestion of the palatable food in this procedure exhibits a progressive increase, with the average rat eating less than half of the ration in the first four sessions. The rats surpass the 50% mark by session 5 to reach a plateau of 75-80% by the end of the procedure.

On PDs 80-81, the rats were food deprived to 82%–85% of their ad libitum weights. No significant differences ($p>0.05$) attributable to the IEA procedure were observed. Mean ad-libitum weights at PD80 were 270.25±4.00 and 279.38±3.52, in ethanol- and vehicle-exposed rats. The 82%–85% level of deprivation was kept across the procedure. On PD83 a habituation session (S) was conducted. The rats were placed in an empty standard housing cage. After 3 min they were returned to the home cage, which was now equipped with a bottle filled with 6% ethanol. The bottle was removed after 90 min and replaced by a water bottle. The rats were then offered a single sugary pill and rat chow.

During the testing sessions (PDs 84-93, S1-10), the rats were weighed and placed in an empty standard housing chamber in which they were exposed to 1 (control group) or 12 (BE group) sugary pills. After 3 min the rats were moved to their home cage and exposed to a single bottle of ethanol for 120 min (concentration: 6% in S1 to S4, 8% in S5-6, and 10% in S7-10; all drinking solutions expressed as %w/w). Before and after each session, the

bottles were weighed to calculate ethanol intake (g/kg). Leakage was accounted for by placing a bottle in an empty cage. The (before – after) readings of that bottle were subtracted from the animal's consumption. We also measured the number of sugary pills eaten by the rats.

A two-bottle (10% ethanol vs. water), 24-h long, intake session (S11) was conducted on PD96. The aim was to assess free choice ethanol drinking (and preference) after the forced adolescent ethanol exposure and the sessions involving, at adulthood, BE and single-bottle ethanol drinking.

Determination of GPx activity, CAT activity, and lipid peroxidation

On PDs 103-104 (see Figure 1) the rats were given 3 administrations of 5 g/kg of ethanol (concentration 33%), every 12 hours. This is a standard model to challenge redox balance (Artun et al., 2010). The rationale was to assess the effect of a history of IEA at adolescence, followed by control or BE conditions, on the antioxidant capacity of the rats.

Whole blood was collected by decapitation, 3 hours after the last administration, using sodium heparin as an anticoagulant. An aliquot was used for hemoglobin determination according to the Drabkin method (Pathirathna et al., 2022). The red cell package was separated from plasma by centrifuging another aliquot at 3500 rpm for 10 min. Plasma was stored at -20°C for lipid peroxidation determination, while red cells were washed three times with isotonic saline and stored at -20°C for CAT and GPx activity measurement. Brain tissue was homogenized in phosphate buffer pH 6.5 and spun at 105000 g for 1 h. The supernatant was stored at -20°C until used. Protein levels were measured by the method of Bradford (Bradford and Ward, 1976) using bovine albumin as standard.

GPx activity was measured using red blood cells hemolyzed using distilled water (1/5 dilution) and centrifuged at 10000 g for 10 min. The supernatant was used to measure enzyme activity according to the method of Paglia and Valentine (1967). Blood and brain GPx activities were determined by measuring NADPH consumption rate per minute during 10 min at 340 nm in a reaction medium containing H_2O_2 as a substrate, and GSH and GSH reductase in excess. One enzymatic unit of GPx was considered as 1 μmol of NADPH oxidized/min and expressed as U/g Hb (blood) or U/gr protein (brain tissue).

The assay for lipid peroxidation measurement (Aguilar Diaz De Leon and Borges, 2020) involved the reaction of lipid peroxidation products, primarily malondialdehyde (MDA), with thiobarbituric acid (TBA) to form thiobarbituric acid reactive substance (TBARS) adducts. Briefly, 100 µl of sample was analyzed under acidic conditions (pH=4) and at 95 °C to form a red-pink product that can be measured spectrophotometrically at 532 nm. Final MDA concentrations were expressed as nmol MDA/mL of plasma or nmol MDA per mg protein (brain tissue).

For CAT activity determination (Aebi, 1984), the red cell package was hemolyzed with distilled water (1/20) and the resulting hemolysate further diluted 1/100 with phosphate buffer pH 7.0. Brain homogenates were also diluted 1/20 and the 1/5 in a similar fashion. Blood and brain CAT activities were assayed by measuring the decrease in H_2O_2 absorbance at 240 nm (ε_{240}=0.0394 $mmol^{-1}$ cm^{-1}) according to the Aebi method. Blood and brain resultant CAT activity was expressed as mmol H_2O_2 decomposed/min/g hemoglobin or mmol H_2O_2 decomposed/min/mg protein.

Statistical analyses

Total distance traveled (cm) in the open field test, the discrimination index in the novel object recognition testing phase and ethanol intake scores (g/kg) in the habituation session were separately analyzed via T tests, with adolescent treatment (IEA or control) as the grouping factor. A RM ANOVA (between group factor: adolescent treatment, object was the within factor) analyzed time spent (s) in the vicinity of objects A and B at the NOR test. The quantity of sugary pills eaten by the rats (proportion from total offered) during S1-S10 was analyzed in BE rats via a RM ANOVA with session as within factor. The analysis was not conducted in Control rats because these ate the single pill given, in almost all sessions.

Ethanol ingested (g/kg) during the binge eating sessions was first analyzed via a RM ANOVA, with adolescent treatment and BE treatment as between factors and sessions 1-10 as the within factor. This omnibus ANOVA yielded a complex set of significant main effects and significant interactions comprising adolescent treatment, BE treatment and sessions. To further explore the loci of these effects and considering that the rats began to eat most of the ration (i.e., showed binge eating) by S5, ethanol intake was separately analyzed in S1-S4 and in S5-S10. Ethanol intake (g/kg and percent preference) and water intake (ml/kg body weight) in the two-bottle (10% ethanol vs. water) test (S11), and all the measures gathered at the biochemical assays were separately analyzed via a factorial ANOVA (between factors: IEA at adolescence and eating conditions at PDs 84-93).

The significant main effects and significant interactions were analyzed via Tukey's *post hoc* test. The alpha level was 0.05. Intake data from two rats was lost at S11 due to technical reasons. Of the 246 biochemical determinations 48 (randomly distributed across the groups) were missed due to technical issues. Data are informed as mean±SEM.

3. Results

OF and NOR tests

Distance travelled (cm) at the habituation phase of the NOR test, and Di scores at the test phase were not affected by the adolescent treatment (t_{39}=1.03, p>0.05; and t_{18}=1.14, p>0.05, respectively). Distance travelled scores were 4603.59±1125.14 and 3461.19±130.74, whereas Di scores were 0.17±0.04 and 0.10±0.04, in ethanol- and vehicle-treated rats respectively. The RM ANOVA showed that the rats preferred the novel vs. the familiar object at test (significant main effect of object: $F_{2,56}$=17.50, p<0.001; η^2p=0.28), yet this measure of recognition memory was not affected by the IEA treatment (data not shown). Similarly, ethanol intake scores (g/kg) at the habituation session were similar in ethanol- and vehicle treated females (0.88±0.15 and 1.01±0.10, respectively; t_{39}=0.69, p>0.05).

Ethanol intake tests

The ingestion of the sugary pills in the BE rats increased as a function of the sessions (significant main effect of session: $F_{9,189}$=34.28, p<0.001; η^2p=0.62, acquisition curve in Figure 2) and was not affected by the IEA treatment. T-tests for a single mean against a reference value of 50% indicated that the rats ingested significantly less than half of the pills in S1-S2 (p<0.05). At S5 there was a trend for the rats to ingest more than 50%, an effect that became significant at sessions 6 to 10 (p<0.05).

FIGURE 2 HERE

The omnibus ANOVA for ethanol intake (g/kg) at the BE sessions indicated significant main effects of IEA and session ($F_{1,37}$=6.35, p<0.05; η^2p=0.15 and $F_{1,37}$=6.64, p<0.05; η^2p=0.15, respectively), and significant interactions between IEA and BE ($F_{1,37}$=6.64, p<0.05; η^2p=0.15), between sessions and IEA ($F_{10,370}$=2.30, p<0.05; η^2p=0.06), and between sessions and BE ($F_{10,370}$=3.13, p<0.001; η^2p=0.08). This complex pattern of

results was further explored by separately analyzing ethanol intake at sessions 1-4 (when consumption of the sugary pellets was less than 50% in BE groups) and at sessions 5-10 (i.e., when the binge eating had been established and the BE rats were eating most of the sugary ration provided).

The ANOVA for ethanol intake (g/kg) at sessions 1-4 (see Figure 3A-B) yielded a IEA × BE interaction, $F_{1,37}=5.53$, $p<0.05$; $\eta^2p=0.13$. The *post-hoc* tests indicated that, among rats given vehicle at adolescence, rats assigned to the BE group consumed slightly, yet significantly, less than Controls. Across groups, ethanol consumption increased significantly ($F_{3,111}=5.20$, $p<0.005$; $\eta^2p=0.12$) at the last session, compared to S1 and S2.

FIGURE 3 HERE

The inspection of Figure 4A-B suggests that at sessions 5 to 10 the level of ethanol ingestion in the group given ethanol at adolescence, and exposed to BE, surpassed that of the other conditions. The ANOVA confirmed these impressions, revealing a significant main effect of IEA and a significant IEA × BE interaction, $F_{1,37}=10.64$, $p<0.005$; $\eta^2p=0.22$ and $F_{1,37}=6.80$, $p<0.05$; $\eta^2p=0.16$, respectively. The *post-hoc* tests revealed that, across the sessions, ethanol intake was significantly greater in the rats given ethanol at the IEA procedure and then exposed to BE, than in any other group. The ANOVA also revealed a borderline BE × session interaction, $F_{5,185}=2.22$, $p=0.05$; $\eta^2p=0.06$, with BE rats exhibiting higher ethanol ingestion than control rats at S9 and S10.

FIGURE 4 HERE

Ethanol intake (g/kg) and preference (%) at the two-bottle choice test conducted on PD96 (S11, Fig. 5) was significantly greater in rats given ethanol at the IEA, compared to the rats given vehicle (significant main effects of IEA; $F_{1,35}=11.77$, $p<0.005$; $\eta^2p=0.25$ and $F_{1,35}=16.34$, $p<0.001$; $\eta^2p=0.32$, respectively). Absolute ethanol intake (g/kg) was also found

to be significantly greater in rats exposed to the BE procedure vs. those exposed to control eating conditions (significant main effect of BE, $F_{1,35}=6.98$, $p<0.05$; $\eta^2p=0.17$, respectively). The interactions between BE and IEA were not significant and water intake was not affected by IEA, BE or its interaction.

FIGURE 5 HERE

GPx activity, CAT activity, and lipid peroxidation determinations

The ANOVAs indicated that none of the biochemical variables under analysis, neither in blood or brain, were significantly affected IEA or BE exposure, or by the interaction between these factors. Descriptive statistics for each variable can be found in Table 1.

TABLE 1 HERE

4. Discussion

The main new finding of the study is the significant combined effect of BE and IEA at adolescence, upon ethanol intake at S1-10 (when the drug was the only fluid available). The repeated exposure to high ethanol dose at adolescence did not alter, when compared to control female rats, ethanol intake at S1-S10. BE, on the other hand, had a mild promoting effect on the ingestion of ethanol. Yet, those rats with a history of adolescent ethanol exposure and an exposure to hypercaloric food, resulting in BE, exhibited heightened and sustained ethanol self-administration, that persisted throughout the BE sessions. This was a sizable effect, with Ethanol-BE rats drinking an average of 2.12 g/kg/120 min in the BE sessions, vs 1.15 g/kg/120 min of the other groups.

An important result is that BE and IEA at adolescence increased, by themselves, ethanol intake at the endpoint, two-bottle drinking assessment, at PD96. Unlike the pattern found at the ethanol-only assessments, there was no interaction between BE and IEA at that test (i.e., at S11, when the rats were given a choice between water and ethanol). It could be

hypothesized that IEA and BE exert synergistic effects only at testing conditions likely to promote binge-like drinking.

Moreover, IEA did not exert significant main effects on ethanol intake, when the drug was the only fluid available (i.e., at sessions 1-10). This lack of changes was not unexpected. The literature on IEA at adolescence, particularly that employing i.p. or i.g. routes of administration, has yielded mixed results concerning this outcome (Towers and Varlinskaya, 2020) and, unlike the present report, has rarely employed females. For instance, (Alaux-Cantin et al., 2013) gave male Sprague-Dawley rats 3.0 g/kg ethanol (i.p.) every 2 days, for 2 consecutive days from PD30 to PD43. This was associated with greater intake of 10% ethanol, measured under continuous access conditions in two-bottle choice tests. Yet (Broadwater et al., 2011), also in males only, found no effect of IEA (4 g/kg every 48 hours, PDs24-33) on intake of 10% ethanol (vs. water, 1h sessions, with ethanol mixed in a sucrose+saccharin solution). Aside from the differences in rat strain, sex and ethanol concentration or vehicle, there are other methodological variations between these reports and the present study. In Broadwater et al. (2011), for instance, the rats were tested shortly after (i.e., 48 hrs.) termination of the IEA protocol. More recently researchers (Towner et al., 2022) gave male and female Wistar rats 4 g/kg ethanol, i.g., every other day on PD25-45 and then tested the rats at PD72-83 for intake of 10% ethanol under social circumstances. This procedure yielded significantly less ethanol intake in IEA vs. control females and either no effect or a mild and transient promoting effect upon ethanol intake in the males. Similarly, and in one of the few studies with Wistars that employed forced administration, the i.p. administration of 2.0 g/kg (PDs 27 to 39) reduced ethanol drinking at PD78, when tested in male rats only in single-bottle, 30-min, tests (Gilpin et al., 2012).

As reviewed, several studies found promoting effects of IEA upon ethanol intake, yet others found no effect, or even less ethanol predilection after IEA. The reasons underlying these mixed effects, at least when using i.p. or ig. routes of ethanol delivery, are mostly unknown. It is possible, however, that the bolus nature of these administration (and the high doses of ethanol employed) favor the development of conditioned aversions towards the taste or odor of ethanol. A minor, yet significant fraction, of ethanol is excreted non-metabolized via urine, feces, and perspiration, and can support ethanol-induced conditioned taste aversions (Dominguez et al., 1994) that preclude the self-administration of significant quantities of ethanol. We can only speculate, but it is possible that the BE and ethanol drinking protocols employed in the present study may have help counteract these effects.

In one hand, in the present study rats were gradually –during the first four sessions- exposed to more concentrated ethanol solutions. These sessions may have allowed the rats to overcome their innate aversion for the taste and odor of ethanol (Walker et al., 2021), and to extinguish any eventual conditioned aversion towards these cues. Moreover, during the first four sessions the BE rats eat less than half of the sugary pills, thus not expressing BE. By session 5 and onwards the rats ate most of the ration in the brief allotted time, thus fulfilling criteria for BE. It is notable that it was at this moment that the Ethanol-BE groups began to show heightened ethanol intake, compared to the other groups. It could be argued that the development of BE "primed" the subsequent ingestion of ethanol, allowing the underlying vulnerability of IEA to be expressed. This speculation is consistent with our prior study in male Wistar rats, in which we found that BE, given immediately before the ethanol drinking session, significantly increased ethanol self- administration (Ruiz-Leyva et al., 2022). The hypothesis is also consistent with models proposing that BE and substance use disorders

share psychological and neurobiological mechanisms (Schreiber et al., 2013) and with studies suggesting priming effects of BE upon ethanol drinking. Rats given intermittent access to sugar displayed signs of sugar dependence (Avena et al., 2004). These rats showed, when tested after discontinuation of the sugar access, heightened intake of unsweetened ethanol, when compared to rats exposed to ad libitum access to sugar. A study conducted in 428 undergraduate students, in turn, showed that food addiction was related to binge drinking, via binge eating (Escrivá-Martínez et al., 2020).

The present results have several limitations, chiefly the absence of neurobiological measurements that pinpoint mechanisms underlying the synergic effects found. IEA is known to induce anxiety (Gilpin et al., 2012) and neural (Trantham-Davidson et al., 2017) alterations likely to promote subsequent ethanol intake; and it was possible that IEA plus BE would have derived in altered antioxidant capacities (Ojeda et al., 2022), which in turn could have promoted ethanol intake. No evidence of these alterations was found in this study. The IEA treatment did not alter exploratory activity nor recognition memory, and IEA+BE did not alter GPx, CAT or lipid peroxidation activity. Another important limitation is that the 3 min binge episode could have affected levels of insulin, glucagon, or other hormones. Supporting this possibility, it has been shown that rats exposed to a sucrose-rich diet (in repeated, 10-min, sessions) exhibited enhanced levels of circulating leptin (Cottone et al, 2008). The limitation in our study could have been circumvented by adding a group administered with an equivalent amount of carbohydrate orally.

Despite its limitations, the present study indicates –to our knowledge, for the first time in female rats- interactive effects between BE and ethanol drinking, that are potentiated by ethanol exposure at adolescence. Ethanol drinking typically begins during adolescence and,

particularly when occurs in a binge-like pattern like the one we modelled via this IEA protocol, can exert lingering adverse consequences (Bravo et al., 2019). The present study suggests that IEA can interact with BE, facilitating the harmful consequences of the ingestion of hyperpalatable foods, upon ethanol intake.

Declaration of absence of conflict of interest: We declare having no competing interest nor conflict of interest related to our MS or its results.

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Figure Legends

Figure 1. Timeline depicting the procedures conducted to assess, in female Wistar rats, interactive effects between intermittent ethanol exposure (IEA) at adolescence (11 every-other-day administrations of 4.0 g/kg ethanol or vehicle, postnatal days 25 to 45, PD25-45) and eating condition at sessions (S) 1 to 10. The rats were given 1 (control eating condition, CEC) or 12 (binge eating group, BE) highly palatable sugary pills to eat in 3 minutes (1.16 or 13.86 kcal, respectively), daily at sessions 1 to 10, beginning at PD84. This was immediately followed by exposure to an ethanol bottle for 120 min (concentration: 6% in S1-4, 8% in S5-6, and 10% in S7-10; %w/w). A 24-h long, two-bottle intake session (10% ethanol vs. water) was conducted on PD96 (S11). On PDs 103-104 the rats were given 3 administrations of 5 g/kg of ethanol, every 12 hours. The rats were sacrificed 3 hours after the last administration, Glutathione Peroxidase (GPx) activity, Catalase (CAT) activity, and lipid peroxidation status were determined in blood and brain.

Figure 2. Female Wistar rats exposed to a binge eating procedure eat, after 5 sessions, most of the ration provided. Quantity of sugary pills eaten (proportion from total offered) during sessions 1 to 10, as a function of intermittent ethanol exposure (IEA) at adolescence (11 every-other-day administrations of 4.0 g/kg ethanol or vehicle, postnatal days 25 to 45, PD25-45) and eating condition at sessions 1 to 10. The rats were given 1 (control eating group) or 12 (binge eating group) highly palatable sugary pills to eat in 3 minutes (1.16 or 13.86 kcal, respectively), daily at sessions 1 to 10, beginning at PD84. The asterisks indicate that, according to single mean T-tests against a constant of 50%, the rats in the binge eating group ingested significantly more than half of the pills in sessions 6 to 10 ($p<0.05$). This analysis was not conducted in control rats, due to them exhibiting near complete ingestion of the ration offered across days. Data are shown as mean±SEM.

Figure 3. A. Intermittent ethanol exposure at adolescence (IEA) does not significantly interact with exposure to highly palatable sugary pills at adulthood.

Ethanol intake (g/kg) during sessions 1 to 4 (postnatal days 84 to 87, PD84-87), as a function of IEA (11 every-other-day administrations of 4.0 g/kg ethanol or vehicle, PD25-45) and eating condition at sessions 1 to 4. The rats, female Wistars, were given 1 (control eating group) or 12 (binge eating group) highly palatable sugary pills to eat in 3 minutes (1.16 or 13.86 kcal, respectively), daily at each session. This was immediately followed by a 120 min exposure to a 6% w/w ethanol bottle. B. During sessions 1 to 4 the consumption of the sugary pellets was significantly less than 50% in the binge eating group. Same data as in panel A yet collapsed across sessions. The asterisk indicates that, among rats given vehicle at adolescence, rats assigned to the binge eating group consumed significantly less ethanol than control eating rats ($p<0.05$). Data are shown as mean±SEM. The combination of both line graphs and bar graphs is meant to facilitate the depiction of significant main effects or significant interactions.

Figure 4. A. Intermittent ethanol exposure at adolescence (IEA) and binge eating at adulthood significantly interact, promoting ethanol self-administration. Ethanol intake (g/kg) during sessions (S) 5 to 10 (postnatal days 88 to 93, PD88-93), as a function of IEA (11 every-other-day administrations of 4.0 g/kg ethanol or vehicle, between PD25-45) and eating condition at sessions 5 to 10. The rats, female Wistars, were given 1 (control eating group) or 12 (binge eating group, BE group) highly palatable sugary pills to eat in 3 minutes (1.16 or 13.86 kcal, respectively), daily at each session. This was immediately followed by exposure to an ethanol bottle for 120 min (concentration: 8% in S5-6, and 10% in S7-10; %w/w). During sessions 6 to 10 the consumption of the sugary pellets was significantly higher than 50% in the BE groups, whereas in session 5 there was a trend for these rats to

ingest more than 50%. B. Same data as in panel A yet collapsed across sessions. The statistical analysis (ANOVA) indicated a significant main effect of IEA (significantly greater ethanol intake in IEA vs. control rats, indicated by the asterisk) and a significant IEA x BE interaction ($p<0.05$). Ethanol intake was, as denoted by the pound sign, significantly greater in the rats given ethanol at the IEA procedure and then exposed to BE, than in any other group. The ANOVA also revealed a borderline BE X session interaction, with BE rats exhibiting higher ethanol ingestion than control rats at S9 and S10. Data are shown as mean±SEM. The combination of both line graphs and bar graphs is meant to facilitate the depiction of significant main effects or significant interactions.

Figure 5. A. Intermittent ethanol exposure at adolescence (IEA) and binge eating at adulthood independently enhance ethanol self-administration at adulthood. Ethanol intake (g/kg), percent ethanol preference vs. water (panels A-B, respectively) and water intake (ml/kg of body weight, panel C) at a two-bottle (10% ethanol vs. water), 24-h long, endpoint intake session conducted on postnatal day 96 (PD96). The data is presented as a function of IEA (11 every-other-day administrations of 4.0 g/kg ethanol or vehicle, between PD25-45) and eating condition at sessions 1 to 10. The rats, female Wistars, were given 1 (control eating group) or 12 (binge eating group) highly palatable sugary pills to eat in 3 minutes (1.16 or 13.86 kcal, respectively), daily at each session. The statistical analysis (ANOVA) indicated that ethanol intake (g/kg) and preference (%) was significantly greater in rats given ethanol at the IEA, compared to the rats given vehicle (significant main effects of IEA; $p<0.05$, indicated by the asterisks). Absolute ethanol intake (g/kg) was significantly greater in rats exposed to the BE procedure vs. rats exposed to control eating conditions (significant main effect of BE, $p<0.05$; indicated by the pound sign). Water intake was not significantly affected by IEA, BE or its interaction. Data are shown as mean±SEM.

Table 1: Measurement of Glutathione Peroxidase (GPx) activity, Catalase (CAT) activity, and lipid peroxidation, in blood and brain.

Enzymatic Activity		IEA Treatment: Vehicle		IEA Treatment: Ethanol	
	Tissue	Control eating	Binge eating	Control eating	Binge eating
GPx	Brain (U/g Prot)	3.65 ± 0.88	4.11 ± 1.23	2.51 ± 0.96	4.96 ± 1.30
	Blood (U/g Hb)	0.46 ± 0.24	0.45 ± 0.13	0.77 ± 0.18	0.45 ± 0.18
CAT	Brain (H_2O_2/min/g Prot)	1635.94 ±466.65	1259.22 ± 444.66	1354.67 ± 378.50	1484.51 ± 446.05
	Blood (H_2O_2/min/g Hb)	4315.93 ± 1204.30	6554.51 ± 952.00	6492.84 ± 1102.79	5940.51 ± 535.69
Lipid peroxidation	Brain ($nmol_{MDA}$/mg Prot)	1.28 ± 0.85	1.04 ± 0.70	1.11 ± 0.83	0.36 ± 0.05
	Blood ($nmol_{MDA}$/ml)	2.27 ± 0.75	1.86 ± 0.22	1.61 ± 0.27	1.37 ± 0.20

Brain and blood determinations of glutathione peroxidase, catalase, and lipid peroxidation in female Wistar rats tested at postnatal days (PDs) 103-104. The data are expressed as mean ± SEM and shown as a function of intermittent ethanol exposure (IEA) at adolescence (11 every-other-day administrations of 4.0 g/kg ethanol or vehicle, PD25-45) and eating conditions (Binge or Control eating) at PDs 84-93. On PDs 103-104 the rats were given 5 g/kg of ethanol, every 12 hours for 3 doses. Blood and brain samples were collected 3 hours after the last administration. GPx activity is expressed as U/g Hb (blood) or U/gr protein (brain tissue). Lipid peroxidation measurement are expressed as nmol MDA/ml of plasma or nmol malondialdehyde per mg protein (brain tissue). CAT activity was expressed as mmol H_2O_2 decomposed/min/g hemoglobin or mmol H_2O_2 decomposed/min/mg protein. The statistical analysis (independent ANOVAs that considered IEA at adolescence and eating conditions at PDs 84-93 as between factors) yielded a lack of significant main effects or significant interactions.

Figure 1

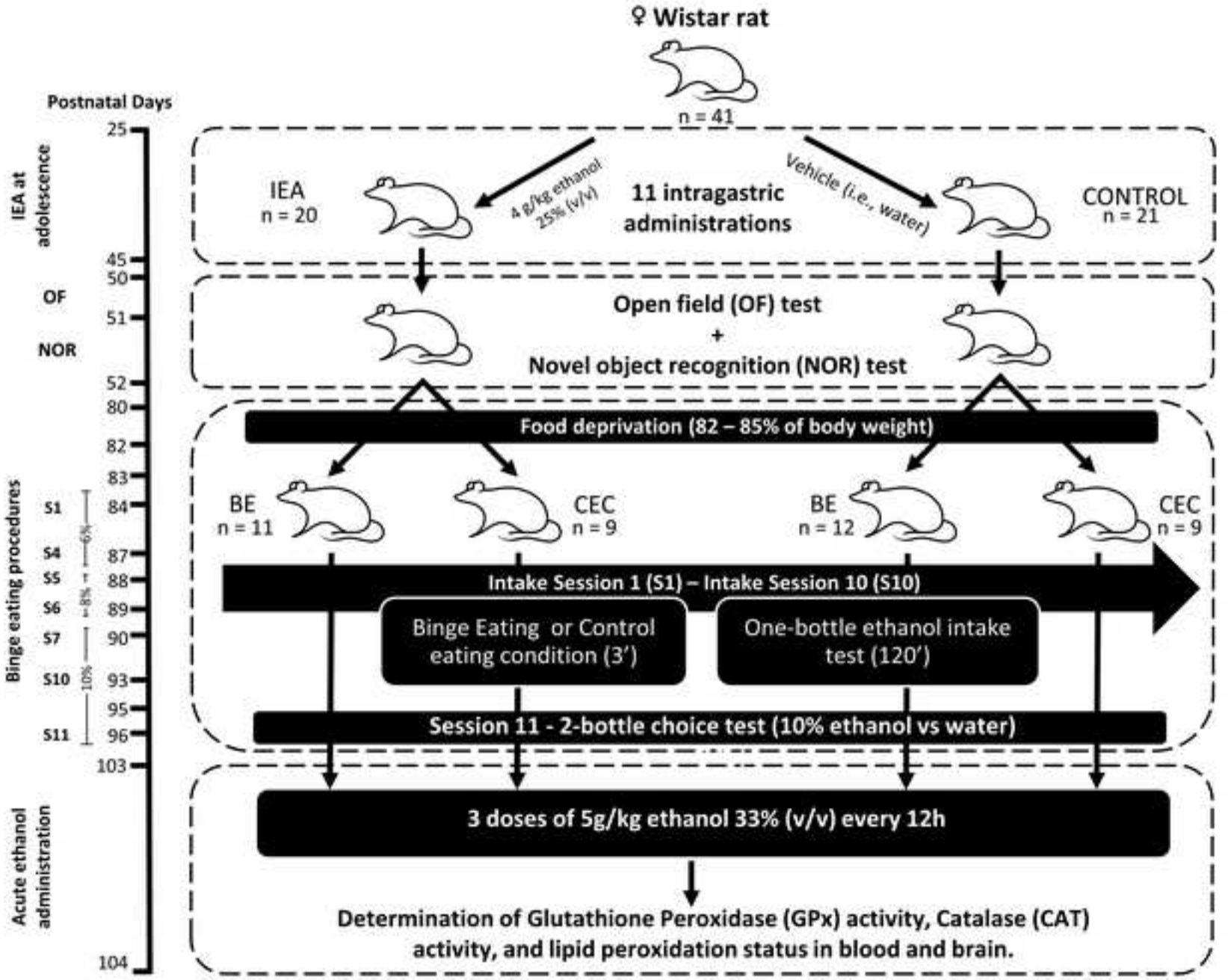


Figure 2

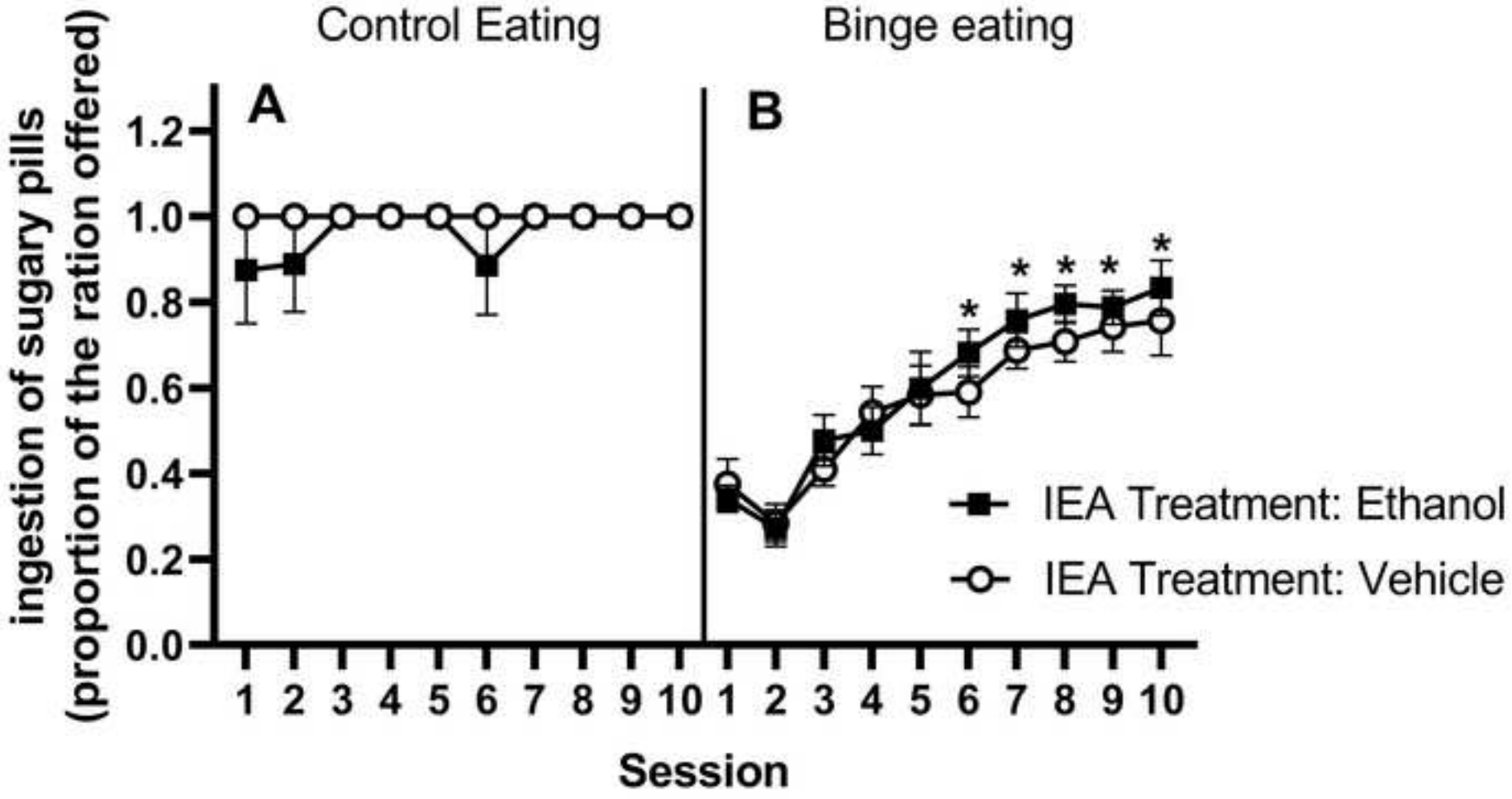


Figure 3

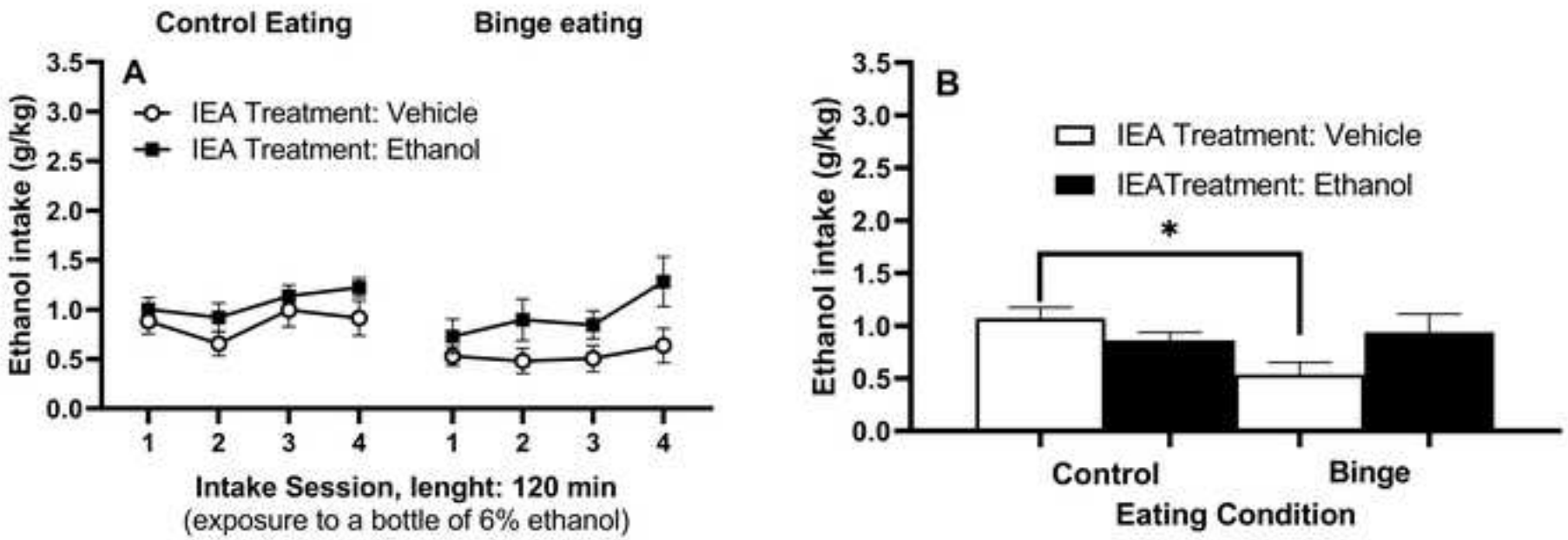


Figure 4

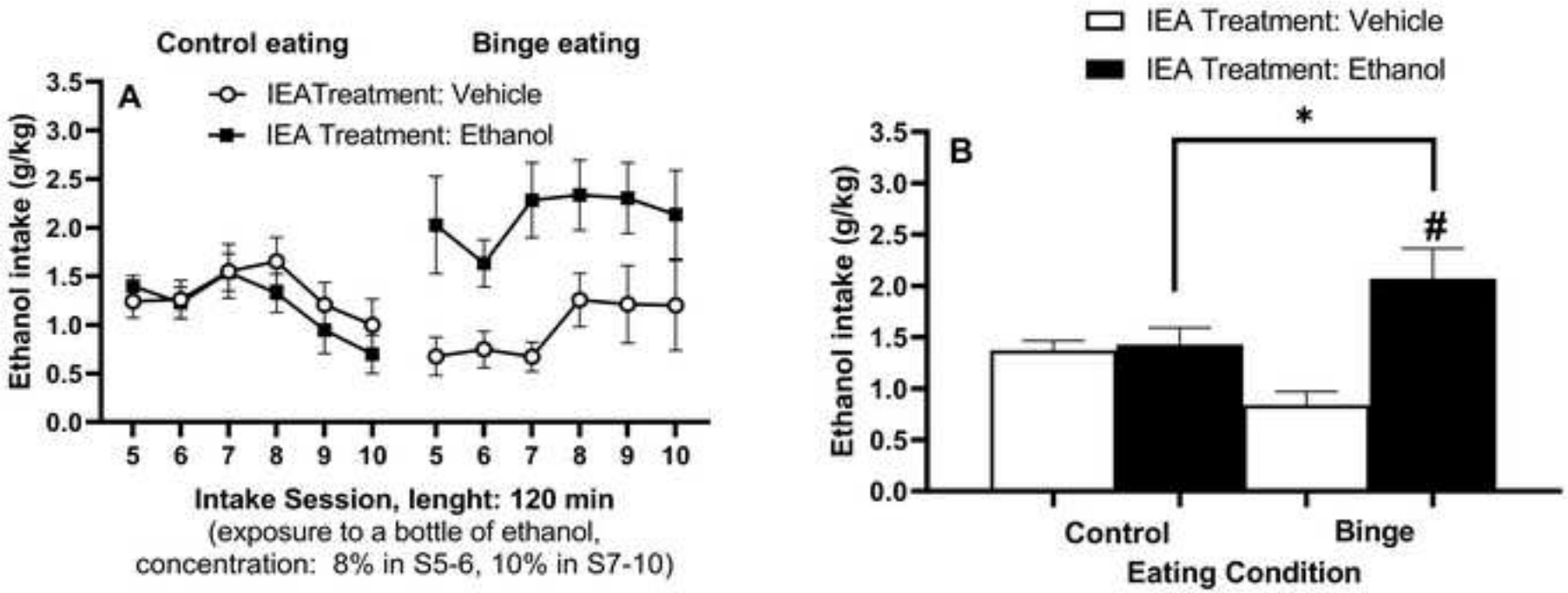
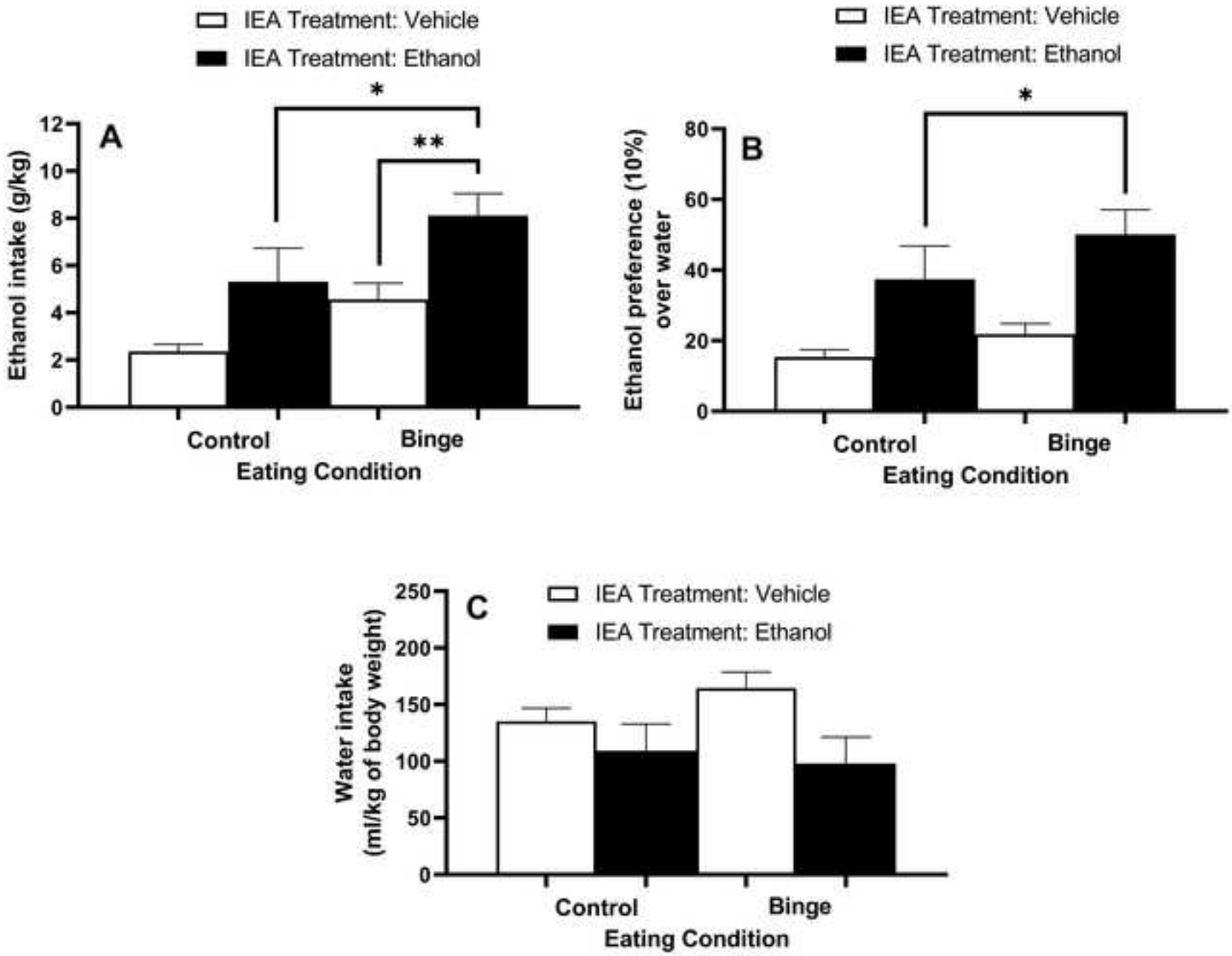


Figure 5



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We declare having no competing interest nor conflict of interest related to our MS or its results.