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Differential rearing alters taste reactivity to ethanol, sucrose, and quinine

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Abstract

Rationale—Early-life environment influences reinforcer and drug motivation in adulthood; however, the impact on specific components of motivation, including hedonic value (“liking”), remains unknown.

Objectives—The current study determined whether differential rearing alters liking and aversive responding to ethanol, sucrose, and quinine in an ethanol-naïve rat model.

Methods—Male and female rats were reared for 30 days starting at postnatal day 21 in either an enriched (EC), isolated (IC), or standard condition (SC). Thereafter, all rats had indwelling intraoral fistulae implanted and their taste reactivity to water, ethanol (5, 10, 20, 30, 40% v/v), sucrose (0.1, 0.25, 0.5 M), and quinine (0.1, 0.5 mM) was recorded and analyzed.

Results—EC rats had higher amounts of liking responses to ethanol, sucrose, and quinine and higher amounts of aversive responses to ethanol and quinine compared to IC rats. While EC and IC rats’ responses were different from each other, they both tended to be similar to SCs, who fell in between the EC and IC groups.

Conclusions—These results suggest that environmental enrichment may enhance sensitivity to a variety of tastants, thereby enhancing liking, while isolation may dull sensitivity, thereby dulling liking. Altogether, the evidence suggests that isolated rats have a shift in the allostatic set-point which may, in part, drive increased responding for a variety of rewards including ethanol and sucrose. Enriched rats have enhanced liking of both sucrose and ethanol suggesting that enrichment may offer a unique phenotype with divergent preferences for incentive motivation.

Keywords

Taste reactivity; Ethanol; Motivation; Hedonic value; Environment; Differential rearing; Enrichment; Post-weaning social isolation; Sucrose; Quinine

Early rearing environments have profound effects on behavior in adulthood. Adverse childhood experiences result in disruptions in reward processing in adulthood (Dennison et

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Compliance with ethical standards

Conflict of interest TJW, ECB, TJM, SWK, and MEC declare that they have no conflict of interest.

al. 2019; McLaughlin and Sheridan 2016). Conversely, enrichment in early childhood reduces drug-related charges in adulthood, when compared to age matched controls (Raine et al. 2003). To understand the neuronal and behavioral changes that result from the early rearing environment, the differential rearing paradigm during the post-weaning period is a widely used animal model (Renner and Rosenzweig 1987; Bennett et al. 1969). The differential rearing paradigm includes an enriched group in which rats are group housed in large cages with novel objects and daily handling during the post-weaning period. The presence of cohorts, contact with novel objects, and the amount of handling are all critical elements that create the enriched environment (Renner and Rosenzweig 1987). Enriched rats are compared to rats reared in an isolated environment consisting of hanging metal cages in which the food and water are changed without handling the rats. These two environmental conditions are often compared to a standard condition (SC) in which rats are housed in pairs without novel stimuli. Social stimulation alone is not sufficient to produce neurobiological changes similar to those observed with enrichment and generally results in neurobiological changes between enriched and isolated housing (Renner and Rosenzweig 1987; Rosenzweig et al. 1978). While neurobiological effects of enrichment are evident after just 4 days of enrichment (Ferchmin et al. 1970), the effects are more consistent and reliable when rats are assigned to their respective environments after weaning and housed in those environments for 30 days (Riege 1971).

Differential rearing has profound effects on operant responding for drug and non-drug rewards. When compared to IC rats, EC rats respond less for a variety of reinforcers including ethanol (Bardo and Dwoskin 2004; McCool and Chappell 2009; Stairs and Bardo 2009; Deehan et al. 2007), psychomotor stimulants (Bardo et al. 2001, T. A. Green et al. 2002, Howes et al. 2000), opiates (M. A. Smith et al. 2005, M. A. Smith et al. 2003), and non-drug rewards (Gill and Cain 2010; Grimm et al. 2008; Rose et al. 1986). These combined results have led to the suggestion that differential rearing alters incentive motivation (Bardo and Dwoskin 2004, Gill and Cain 2010, Beckmann and Bardo 2012, Kirkpatrick et al. 2013).

Incentive motivation is comprised of the motivation to respond for the reward (“wanting” or “incentive salience”) and the hedonic value of the reward (“liking”) (Robinson and Berridge 2003; Berridge and Robinson 2011; Robinson and Berridge 2008; Berridge and Robinson 2016). It is well established that the EC reduces the wanting for a variety of rewards while the IC increases the wanting for a variety of rewards using operant paradigms (Bardo and Dwoskin 2004; Bardo et al. 2013; Simpson and Kelly 2011; Stairs and Bardo 2009). IC rats display enhanced reward discrimination when compared to EC or SC rats (Kirkpatrick et al. 2013). In addition, EC rats engage in more goal tracking whereas IC rats engage in more sign tracking in a Pavlovian conditioned approach task (Beckmann and Bardo 2012), suggesting that enrichment decreases the attribution of incentive salience to rewards. Enriched environments may protect against drug use and abuse by reducing the incentive salience of drug rewards (Stairs and Bardo 2009) and the incentive value of stimuli that are paired with rewards (Beckmann and Bardo 2012). Conversely, the isolated environment may result in increased sensitivity to rewards and reward-related stimuli due to enhanced incentive salience to rewards (Beckmann and Bardo 2012, Brenes and Fornaguera 2008, Morgan and Einon 1975, Wood et al. 2006).

While it is clear that differential rearing alters incentive salience or wanting, it is not clear how differential rearing alters liking. The majority of work examining the effects of differential rearing has used operant paradigms. It is difficult to separate the components of incentive motivation using operant paradigms (Samson et al. 2003; Samson et al. 1998). Interestingly, when ethanol consumption is measured using non-operant paradigms, enrichment actually increases ethanol intake when compared to isolated rats (Rockman et al. 1989; Rockman et al. 1986). In addition, several other experiments have observed no effect of isolation on ethanol consumption and/or preference when compared with enrichment or standard housing (Chappell et al. 2013; Deatherage 1972; Kazmaier et al. 1973; Rockman and Gibson 1992). However, experiments that measure ethanol consumption are confounded by the effects of the taste of ethanol and the post-ingestive consequences of ethanol (Kiefer 1995; Kiefer and Dopp 1989). It is possible that differential rearing is altering the incentive salience or wanting to consume ethanol, as evidenced by the consistent effects of differential rearing on operant responding for ethanol but that differential rearing is not consistently altering the liking of ethanol, as evidenced by the discrepancies across numerous consumption experiments. Therefore, it is essential to determine the effects of differential rearing on the hedonic response to ethanol and other reinforcers using the taste reactivity paradigm.

Taste reactivity (TR) can be used to quantify liking of a substance. During TR orofacial behavioral reactions are recorded and tallied as the rats react to various tastants infused intraorally through an implanted fistula. The tallied behaviors fall into categories of hedonic (liking) or aversive (disliking). Sucrose, a palatable tastant, tends to elicit almost exclusively hedonic orofacial behaviors; whereas quinine, an unpalatable bitter tastant, tends to elicit predominantly aversive orofacial behaviors (Grill and Norgren 1978; Kiefer et al. 1990; Berridge 2000). These typical reactions to palatable and unpalatable tastants as well as the neural substrates of these behaviors indicate that taste reactivity measures liking without engaging wanting (Berridge 2000). Shifts in taste reaction behaviors parallel shifts in hedonic neural activity that are related to learning, memory, and stimulus value demonstrating that taste reactivity can indeed be used as a measure of liking (Berridge et al. 1981; Delamater et al. 1986; Berridge and Schulkin 1989; Kerfoot et al. 2007; Castro and Berridge 2014; Peciña and Berridge 2005; Holland et al. 2008).

In addition to sucrose and quinine, another widely studied tastant is ethanol (for review see: Kiefer 1995, Lemon et al. 2004, Lemon et al. 2011). Previous research examining taste reactivity to ethanol found that ethanol tends to elicit a combination of both hedonic and aversive reactions. As the amount of previous experience with ethanol increases, aversive taste reactions to ethanol decrease (Kiefer and Dopp 1989) and hedonic responses increase (Kiefer et al. 1994). Genetic influences on TR to ethanol have also been examined among rats selectively bred for ethanol preference (Preferring [P] and Non-preferring: [NP] rats Bice and Kiefer 1990) and ethanol drinking (High and Low Alcohol Drinking [HAD/LAD] rats: Kiefer et al. 1995). These studies found that among genetically vulnerable populations, including the P and HAD rats, ethanol experience decreased aversive responding and increased hedonic responding overall with a pattern of escalating hedonic responses as ethanol concentration increased (Bice and Kiefer 1990). However, previous research found no differences between taste reactivity among these genetically vulnerable strains when they

were alcohol naïve (Bice and Kiefer 1990; Kiefer et al. 1995) and no relationship between taste reactivity to alcohol in naïve rats and later alcohol consumption (Bice et al. 1992; Kiefer and Dopp 1989). While genetically induced and ethanol experience-mediated differences in ethanol liking have been established, how early-life environment may affect ethanol liking remains interesting and unanswered given what is known about its effects on incentive salience.

Therefore, the purpose of the present study was to determine whether differential rearing alters liking of ethanol, sucrose, and quinine. EC rats respond less in operant and consumption paradigms for ethanol (Deehan et al. 2007; Deehan et al. 2011) and sucrose (Hall et al. 1998; Brenes and Fornaguera 2008) than SC and IC rats. Therefore, it was hypothesized that EC rats would have decreased liking and show less hedonic responding than IC rats for ethanol and sucrose. Alternatively, EC rats may be more sensitive to the rewarding effects of drug and non-drug rewards due to the increased turnover of dopamine (DA) in the mesocorticolimbic system (Stairs and Bardo 2009; Zhu et al. 2004) and this change in DA functionality may reduce their incentive motivation, but not their liking responses. If this hypothesis is supported, EC rats will have similar or more hedonic responding than IC rats for sucrose and ethanol.

Materials and methods

Animals and rearing conditions

Seventy-six male ($n = 40$) and female ($n = 36$) Long Evans rats (Charles River Laboratories) arrived in the lab at postnatal day (PND) 21 and were randomly assigned to rear in one of three environmental conditions: enriched (EC; $n = 30$), isolated (IC; $n = 24$), or standard (SC; $n = 22$) conditions for 30 days using methods previously described (Deehan et al. 2011; Arndt et al. 2019). ECs were group housed (8–10 per cage) in a large metal cage ($60 \times 120 \times 45$ cm) lined with bedding. The EC cage contained 14 hard plastic objects (e.g., commercially available children's toys, PVC pipe, plastic containers, etc.). Seven of the objects were exchanged and all objects were rearranged into a novel configuration daily. ECs were removed daily for approximately 1 min of experimenter handling. ICs were individually housed in hanging metal cages ($17 \times 24 \times 20$ cm) with steel sides and a wire mesh front and bottom and were not handled during the rearing period. SC rats were briefly handled during weekly bedding changes and housed two per standard shoebox cage ($20 \times 43 \times 20$ cm) with a wire rack top and bedding. After the rearing period, rats remained in their respective environments for the duration of the study. All rats were housed under a standard 12-hour dark/light cycle at a temperature of 21 ± 1 °C and humidity between 30 and 50% along with ad libitum food and water access throughout the experiment. This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (National Research Council 2011) and was approved by the Institutional Animal Care and Use Committee of Kansas State University.

Drugs and chemicals

Ethanol, 200 proof (Decon Lab, Inc.; King of Prussia, PA, USA), was prepared with deionized water to concentrations specified below. Quinine HCl (Tocris Bioscience; Bristol, UK) and sucrose were dissolved in deionized water to specific concentrations detailed below. Ketamine (60–80 mg/kg; 1 mg/ml, i.p.) and diazepam (5 mg/ml; 1 mg/ml, i.p.) were diluted to dose-specific concentration in sterile normal saline. Isoflurane gas (1–3%, i.h.; Akorn Animal Health, Lake Forest, IL) was used during anchoring of the fistula and head mount to the skull.

Taste reactivity

At approximately PND 65, rats were deeply anesthetized with ketamine and diazepam and were maintained with isoflurane. An intraoral fistula made of polyethylene tubing (PE-60; Instech Labs; Plymouth Meeting, PA) was implanted unilaterally, anterolateral to the first maxillary molar and threaded subcutaneously to exit on top of the skull. The tubing was connected to a head mount (313–000/SPC, PlasticsOne, Roanoke, VA) and both were secured to the skull with screws and dental acrylic. Fistulas were flushed with sterile water daily to maintain viability.

Following recovery from surgery, all rats began habituation sessions. At the start of each session, the rat was tethered to the leash and the swivel ensured free movement of the rat in the chamber. After 3 habituation sessions (1.5 min each), each rat received one test session (one solution) daily and orofacial movements were recorded. In the event the data from a test session was unusable (e.g., syringe pump did not work, rat became disconnected from the tether), the remaining test solution was re-tested prior to the final water trial. Taste reactivity to water, ethanol (5, 10, 20, 30, and 40% v/v), sucrose (0.10 M, 0.25 M, and 0.50 M), and quinine HCl (0.1 mM and 0.5 mM) were performed in an order determined by a partial Latin square so that each rat received each substance concentration once (see Fig. 1 for an illustrative example). Once the orders were determined, animals from each group were yoked to each order to counterbalance any order effects across groups. Water trials were always both the first and last trials in every order. After testing occurred, rats were returned to their home cages.

Taste reactivity testing occurred during the light cycle, in a tall (27 cm) trapezoidal chamber (Base widths 27 cm front × 7.25 cm back × 18.55 cm sides; Top widths 14.6 cm front × 8 cm back × 6.10 cm sides). The chamber had clear polycarbonate front and floor panels and three mirrored interior walls tapering in towards the top of the chamber and angled at approximately 70° from the floor of the chamber. A mirror was placed below the chamber angled at approximately 45° toward the front and floor. A high-definition video camera (1080p @ 59.94 frames per second; Cannon Vixia HF R800) faced the chamber with all visible angles typically within the camera frame (Fig. 2). Solutions were administered via a syringe pump (78–0100, KDScientific, Holliston, MA) connected to a 10-mL syringe. Solutions were infused at a rate of 1 mL/min through a leash and swivel arrangement comprised of a polyethylene supply tube encased in vinyl tubing with a captive collar to secure the unit to the head mount (Plastics One; Roanoke, VA).

Rats' orofacial responses during each taste reactivity session were scored frame-by-frame by trained raters blind to the experimental conditions of the animals using BORIS (Behavioral Observation Research Interactive Software; Friard and Gamba 2016). A single trial consisted of 1.5 min of infusion with a scoring time of 1 min beginning after the first visible orofacial response. The specific responses recorded were categorized using criteria previously described in the literature as either hedonic (mouth movements, tongue protrusions, and lateral tongue protrusions) or aversive (gapes, passive drips, head shakes, forelimb flails, fluid expulsions, chin rubs, and paw pushes; Kiefer 1995, Spector et al. 1988, Grill and Norgren 1978).

Data analysis

All data was analyzed using JMP 12.1 (SAS Institute Inc., Cary, NC). To determine whether differences in taste reactions existed between environmental groups (EC, IC, and SC), separate linear mixed effects models (i.e. repeated measures regressions) were performed on each class of taste reaction behaviors (hedonic or aversive) for each substance (ethanol, sucrose, or quinine) with the full factorial fixed (main) effects of Concentration and Environment and the random effect of the intercept to create a repeated measures analysis. The random effect of the interaction between the intercept and Concentration was included where indicated when enough data for each animal at multiple concentrations was available. The inclusion of the interaction between the intercept and Concentration was included when possible to improve estimation of the rate of change across concentrations of a given substance. To determine whether the response to a neutral water tastant differed between environmental groups across time, separate linear mixed effects models were performed on each class of taste reaction behaviors with the full factorial fixed (main) effects of Session (initial and final water session as categories) and Environment along with the random effect of the intercept to create a repeated measures analysis.

Bonferroni-corrected multiple comparisons and simple slopes analyses were performed where indicated and the alpha-level was set to 0.05 for all analyses.

Interrater reliability was analyzed using separate Pearson's correlations between each pair of raters' totals for each class of taste reaction behaviors (hedonic or aversive) across several randomly selected videos and time intervals from videos. The average correlation for hedonic reactions was high (average $r = .91$) along with the correlations of aversive reactions between raters (average $r = .88$), suggesting a high level of consistency between raters across substances and environmental conditions.

Results

All models were initially run including the fixed (main) effect of sex and full factorial interactions with sex. The sex effect, and all interactions with sex, were not significant and therefore were excluded in all analyses to simplify results and data visualization.

Ethanol

Hedonic reactions—Overall, the results reveal that EC rats had higher overall hedonic responses compared to IC rats and a decline in hedonic responses as concentration increased

compared to SC rats. Analysis of hedonic reactions to ethanol included the random effect of the interaction between intercept and Concentration. The analysis of hedonic reactions to ethanol ($R^2 = .66$, $R^2_{\text{Adjusted}} = .66$, $p < .05$; Fig. 3a, b) revealed no fixed (main) effect of Concentration ($p > .05$). The fixed effect of Environment was significant ($F(2,55) = 6.88$, $p = .002$), with post hoc analyses indicating that ethanol elicited significantly more hedonic responses in ECs when compared to ICs, $F(1,56) = 13.76$, $p < .001$. The interaction of Concentration and Environment was significant, $F(2,53) = 4.66$, $p = 0.014$. Bonferroni-corrected post hoc analysis of the simple slopes showed that ECs have a significantly greater rate of decline in hedonic responses as ethanol concentration increases compared to SCs ($F(1,55) = 8.62$, $p = .005$; EC: $b = -2.08$, SC: $b = 0.59$). The comparison of the rate of change between ECs and ICs ($b = 0.12$) did not survive Bonferroni correction ($F(1,53) = 4.66$, $p = .036$). These results suggest that differential rearing affects both overall hedonic responding to ethanol as well as rate of change in hedonic responding across clinically relevant ethanol concentrations.

Aversive reactions—In general, EC rats also had higher levels of aversive responses to ethanol compared to IC rats but rearing conditions did not influence the positive relationship between ethanol concentration and aversive behaviors. Analysis of aversive reactions to ethanol included the random effect of the interaction between intercept and Concentration. The analysis of aversive reactions to ethanol ($R^2 = .69$, $R^2_{\text{Adjusted}} = .68$, $p < .05$; Fig. 3c, d) revealed a significant fixed (main) effect of Concentration, $F(1,47) = 19.01$, $p < .001$, $b = 0.47$, such that every 1% increase in ethanol concentration produced an increase of 0.47 aversive taste reactions. The fixed effect of Environment was significant, $F(2,46) = 3.46$, $p = 0.040$. Post hoc analyses indicate ECs had significantly greater aversive taste responses to ethanol compared to ICs, $F(1,46) = 6.82$, $p = 0.012$. The interaction of Concentration and Environment was not significant ($p > .05$), suggesting that differential rearing influences overall aversive responding but does not influence the rate of change in aversive responding as ethanol concentration increases.

Sucrose

Hedonic reactions—EC rats displayed more hedonic responses to sucrose than IC rats. However, rearing environment did not impact the positive relationship between hedonic responding and sucrose concentration. The analysis of hedonic reactions to sucrose ($R^2 = .50$, $R^2_{\text{Adjusted}} = .49$, $p < .05$; Fig. 4a, b) revealed a significant fixed (main) effect of Concentration, $F(1,106) = 8.77$, $p = .004$, $b = 118.63$, such that every 0.1 M increase in sucrose concentration produced an increase of 11.86 hedonic taste reactions. The fixed effect of Environment, $F(2,57) = 3.75$, $p = .030$, was significant. Post hoc analyses indicated sucrose elicited significantly more hedonic responses in ECs when compared to ICs, $F(1,58) = 7.50$, $p = .008$. The interaction of Concentration and Environment was not significant ($p > .05$). These results indicate that differential rearing affects overall hedonic responding to appetitive sucrose stimuli but does not influence the rate of change in hedonic responding as sucrose concentration increases.

Aversive reactions—Analysis of aversive reactions to sucrose included the random effect of the interaction between intercept and Concentration. As expected, the number of aversive

responses to sucrose was quite low and was not influenced by concentration or rearing environment. The analysis of aversive reactions to sucrose ($R^2 = .44$, $R^2_{\text{Adjusted}} = .42$, $p > .05$; Fig. 4c, d) revealed no significant main effects or interactions between Concentrations or Environments (all $ps > .05$) suggesting neither Concentration nor Environment influenced aversive responding to sucrose.

Quinine

Hedonic reactions—The results reveal a negative relationship between hedonic responding and quinine concentration, but this relationship was not influenced by rearing environment. In addition, EC rats had greater hedonic responding to quinine compared to IC rats. The analysis of hedonic reactions to quinine ($R^2 = .57$, $R^2_{\text{Adjusted}} = .55$, $p < .05$; Fig. 5a, b) revealed a significant fixed (main) effect of Concentration, $F(1,51) = 6.86$, $p = 0.012$, $b = -57.85$, such that every 0.1 mM increase in quinine concentration produced a decrease of 5.78 hedonic taste reactions. The fixed effect of Environment was also significant, $F(1,54) = 4.25$, $p = .019$. Post hoc analyses indicate ECs had significantly greater hedonic taste responses to quinine compared to ICs, $F(1,54) = 7.35$, $p = .009$. The comparison of ECs and SCs did not survive Bonferroni correction ($F(1,54) = 5.10$, $p = .028$). The interaction of Concentration and Environment was not significant ($p > .05$). Together, these results show that differential rearing influences overall hedonic responding to aversive quinine stimuli but does not influence the rate of change in hedonic responding as quinine concentration increases.

Aversive reactions—Analyses revealed a positive relationship between aversive responding and quinine concentration, but this relationship was also not influenced by rearing environment. In addition, EC rats had greater aversive responding to quinine compared to IC rats. The analysis of aversive reactions to quinine ($R^2 = .57$, $R^2_{\text{Adjusted}} = .55$, $p < .05$; Fig. 5c, d) revealed a significant fixed (main) effect of Concentration, $F(1,45) = 4.29$, $p = 0.044$, $b = 16.38$, such that every 0.1 mM increase in quinine concentration produced an increase of 1.64 aversive taste reactions. The fixed effect of Environment was also significant, $F(2,49) = 4.28$, $p = 0.019$. Post hoc analyses indicate ECs had significantly greater aversive taste responses to quinine compared to ICs, $F(1,49) = 7.54$, $p = .008$. The comparison of ECs and SCs did not survive Bonferroni correction ($F(1,49) = 5.19$, $p = .027$). The interaction of Concentration and Environment was not significant ($p > .05$) suggesting that differential rearing influences overall aversive responding to aversive quinine stimuli but does not influence the rate of change in aversive responding as quinine concentration increases.

Water

Hedonic reactions—Hedonic responding to water on the initial water trial was similar across all groups. However, EC and SC rats increased their hedonic responding from the first to the second water trial and had more hedonic responses than ICs during the second trial. The analysis of hedonic reactions to water ($R^2 = .56$, $R^2_{\text{Adjusted}} = .53$, $p < .05$; Fig. 6a, b) revealed a significant fixed (main) effect of Session, $F(1,65) = 14.97$, $p < .001$, where the initial water session had less hedonic reactions than the final water session. The fixed effect of Environment was also significant, $F(2,80) = 3.65$, $p = .031$, but was qualified by the

significant interaction of Session and Environment, $F(2,65) = 5.64$, $p = 0.006$. Post hoc indicated ECs and SCs, $F(1,66-67) = 7.06-17.90$, $ps < .017$, significantly increased their hedonic responding from the initial water session to the final water session. ECs and SCs responded more during the final water session than ICs, $F(1,100-101) = 6.30-12.45$, $ps < .017$. These results suggest that differential rearing affects overall hedonic responding to water during the final but not the initial water session.

Aversive reactions—Aversive responding to water on the initial water trial was similar across all groups. Similar to hedonic responses, aversive responses increased from the first to the second water trial; however, these increases did not differ according to environment. The analysis of aversive reactions to water ($R^2 = .39$, $R^2_{\text{Adjusted}} = .36$, $p < .05$; Fig. 6c, d) revealed a significant fixed (main) effect of Session, $F(1,70) = 11.59$, $p = .001$, such that the final water session had more aversive responses than the initial water trial. The fixed effect of Environment and the interaction of Session and Environment were not significant ($ps > .05$) indicating that aversive behaviors increased from the initial to the final water session but aversive responding to water was not dependent on Environment.

Discussion

The results revealed that differential rearing alters taste reactivity to ethanol, quinine, and sucrose solutions. Surprisingly, enrichment increased hedonic responses across a range of sucrose concentrations and also increased aversive responses across a range of ethanol and quinine concentrations when compared to isolated rats. Conversely, isolation reduced hedonic and aversive responses and appeared to reduce sensitivity to the different concentrations of sucrose and quinine. These results suggest that environmental enrichment may enhance sensitivity to a variety of tastants, thereby enhancing liking, while isolation may dull sensitivity, thereby dulling liking.

Notably, much of the early taste reactivity research with ethanol was performed with rats housed in cages similar to the isolated rats in the current study. However, rats in previous studies were handled frequently and were not isolated during adolescence (e.g. Kiefer and Badia-Elder 1997) which considerably attenuates the isolation effect (Walker et al. 2019; Holson et al. 1991; Pritchard et al. 2013; Fone and Porkess 2008). Thus, it is unsurprising that standard condition rats show similar hedonic and aversive responding that is comparable with findings among ethanol-naïve rats of previous studies in other strains (Kiefer and Dopp 1989; Bice and Kiefer 1990; Kiefer and Badia-Elder 1997). Interestingly, enriched rats' hedonic responding declined as ethanol concentration increased — hedonic responding in standard rats in previous research typically remains flat across concentrations or increases slightly (e.g. Kiefer and Badia-Elder 1997, Bice et al. 1992).

Environmental enrichment is hypothesized to protect against drug use and abuse by reducing the incentive salience of drug rewards (Stairs and Bardo 2009) and the incentive value of stimuli that are paired with rewards (Beckmann and Bardo 2012). Conversely, isolation is hypothesized to increase sensitivity to rewards and reward-related stimuli due to enhanced incentive salience of rewards (Beckmann and Bardo 2012, Brenes and Fornaguera 2008, Jones et al. 1990, Morgan and Einon 1975, Wood et al. 2006). Surprisingly, our current

results suggest that isolation decreases or dulls the hedonic value of ethanol and sucrose. Examination of hedonic responding across the five concentrations of ethanol and three concentrations of sucrose reveals a relatively flat slope in the isolated rats, suggesting they cannot detect differences in hedonic value across a wide range of concentrations.

The inability of isolated rats to detect differences in hedonic value may be due to a shift in their hedonic set-point. Post-weaning social isolation results in a dysfunctional negative feedback loop within the hypothalamic pituitary adrenal (HPA) axis (Serra et al. 2005; Weiss et al. 2004; Stairs et al. 2011). This results in an increased expression of anxiety, startle, and anhedonia-like behaviors in IC rats (Fone and Porkess 2008). This negative emotional state is associated with increased drug-taking behavior and an increase in the rewarding effects of drugs (Shaham et al. 2000; Koob 2008; Covington and Miczek 2001). Based on our current results and the previous literature, isolation shifts the “hedonic set point” resulting in reduced responsiveness to rewarding stimuli (Ahmed and Koob 1998; Solinas et al. 2010) and results in phenotype similar to that hypothesized to be characteristic of sensitization of incentive salience (Berridge et al. 2009, 2003, Robinson and Berridge 1993, 2008). Consistent with the opponent process theory (Solomon and Corbit 1974), isolation increases tolerance to cocaine during extended access (Gipson et al. 2011). Therefore, post-weaning social isolation may be a pre-clinical model of allosteric regulation not caused by prolonged exposure to psychostimulants and may offer an opportunity to examine the neural mechanisms of an allostatic-like phenotype in a drug-naïve model system. Further, while our results indicate that isolation results in low amounts of liking, a large amount of previous literature indicates isolation results in high amounts of wanting (Bardo and Dwoskin 2004; Bardo et al. 2013; Simpson and Kelly 2011; Stairs and Bardo 2009), and therefore offers a pre-clinical model with divergent outcomes for incentive motivation.

While isolation attenuated the hedonic value of ethanol and sucrose, enrichment increased the hedonic value for all substances tested as compared to isolated rats. This result was also surprising given the extensive literature suggesting that enrichment reduces incentive motivation (Bardo and Dwoskin 2004, Beckmann and Bardo 2012, Gill and Cain 2010, Kirkpatrick et al. 2013). The current results suggest that while enrichment predominantly reduces the incentive salience (wanting) component of incentive motivation, it increases both hedonic and aversive behaviors. This unique behavioral profile may be due to the neurobiological changes that result from environmental enrichment. The neurobiological circuits for wanting are widely distributed within the mesocorticolimbic circuits and are predominantly mediated by midbrain dopamine projections to the forebrain (Berridge 1996; Berridge 2009; Berridge and Kringelbach 2013; Berridge and Robinson 1998; Robinson and Berridge 2003). Conversely, liking is controlled by a collection of small hedonic hotspots within larger structures predominately within the limbic system (Berridge 2019; Peciña and Berridge 2005; Peciña et al. 2006; Richard et al. 2013). Interestingly, while these hotspots can enhance liking reactions when stimulated with opioids and other neurotransmitters, dopamine fails to enhance liking (Berridge and Kringelbach 2013; Berridge et al. 2009; Peciña and Berridge 2000; Peciña and Berridge 2005; Peciña and Berridge 2013). The role of dopamine appears to be restricted to wanting (Berridge and Kringelbach 2013, Berridge 2007, K. S. Smith and Berridge 2005). While enrichment does not alter basal levels of dopamine, it does alter functional activity and turnover of dopamine within the

mesocorticolimbic circuit (Bardo and Hammer 1991, Bardo et al. 1995, Solinas et al. 2009, Stairs and Bardo 2009, Zhu et al. 2004). Therefore, enrichment-induced changes to dopaminergic function may explain why divergent outcomes are observed when examining liking versus wanting.

Alternatively, the effects in enriched rats could partially be explained by an enhanced novelty response in enriched rats. While enriched rats habituate more readily to novelty (Cain et al. 2006; Zimmermann et al. 2001), they approach novelty more quickly than isolated rats (Fuller 1967; Renner and Rosenzweig 1986; Widman and Rosellini 1990). Given that all the concentrations of substances tested were novel and only tested once per rat for 1 min, the enhanced responding could be due to enhanced exploration with novel stimuli. This hypothesis is partially supported by the lack of differences between enriched and isolated rats during the first water session. We used the same water source during the session as was used when flushing the fistulas daily and therefore it is not novel. However, the fact that the enriched rats increased hedonic responding across the two water sessions does not support this hypothesis and suggests future experiments are required to determine if the enhanced reactions in enriched rats are due to enhanced liking, enhanced novelty detection, or a combination of factors.

While results from experiments using operant paradigms have consistently observed that enrichment decreases incentive salience, the results from consumption paradigms have been inconsistent (Chappell et al. 2013; Rockman et al. 1989). Interestingly, consumption of ethanol and taste reactivity responses to ethanol are not correlated (Bice et al. 1992), suggesting the two measures are mediated by different factors. The taste of ethanol is complex and intake is mediated by numerous factors including palatability, post-ingestive effects, associative learning, fluid balance, and motivation (Kiefer 1995). In the present study, environmentally-induced differences in taste reactivity were found in naïve animals. Interestingly, taste reactivity to ethanol between P (alcohol Preferring) and NP (alcohol Non-preferring) rats is not different in naïve animals (Bice and Kiefer 1990). In P rats, sweet-sensitive and selective neurons within the nucleus of the solitary tract respond more to ethanol among ethanol-naïve rats when compared to Wistar controls (Lemon et al. 2011). This difference in pattern of reactions to ethanol between naïve P and NP rats and EC and IC rats suggests different hedonic mechanisms are at play between ethanol experience and early-life environment-induced changes in taste reactivity.

The pattern of hedonic sucrose responses along with hedonic and aversive quinine responses were in line with previous research (Ferraro et al. 2002). Higher sucrose concentrations had higher hedonic responses with little to no aversive responses. Higher quinine concentrations had lower hedonic responses and higher aversive responses. Altogether, this suggests that the taste reactivity methods were sensitive to changes in hedonic value that come with changes in substance concentrations. While the direction of effects across concentrations was as expected, the lack of a sex effect was interesting. The literature concerning sex and sex hormone-related differences has focused primarily on intake and preference for sweet and bitter solutions in the past. When differences in intake and preference for saccharin and quinine solutions have been found between males, and intact, ovariectomized or androgenized females, the effects were small or mixed (Wade and Zucker 1970; Nance et al.

1976). In addition, absolute consumption (mL) was used to measure intake which does not control for differences in body size (Wade and Zucker 1970; Nance et al. 1976). Previous research on sex differences that directly examined taste reactivity is even more limited and one study found females in diestrus/proestrus had higher hedonic and lower aversive responding compared to males. However, that result is an average across one concentration of sucrose, quinine, and a sucrose-quinine mixture and to our knowledge there are no experiments examining sex differences and taste reactivity to ethanol (Clarke and Ossenkopp 1998). While the current results suggest no sex differences in taste reactivity exist across a range of sucrose, quinine, and ethanol concentrations, we did not directly measure estrus cycle on each test session to determine if an estrus phase may be influencing taste reactivity to one or all of the substances.

There is less research examining the effects of enrichment on sucrose consumption, but it suggests that enriched rats consume less sucrose than isolated rats (Brenes and Fornaguera 2008). While taste reactivity to sucrose and consumption of sucrose may change across the lifespan, no papers have directly explored if taste reactivity predicts sucrose consumption (Wilmouth and Spear 2009). When observing the underlying neural substrates of the relationship between ethanol and sweet tastants (e.g., sucrose), evidence suggests that ethanol activates sucrose sensitive parts of the gustatory system (Lemon et al. 2004; Lemon et al. 2011). Given the lack of relationship between taste reactivity in ethanol-naïve rats and ethanol consumption (Kiefer and Dopp 1989), additional research is necessary to determine this relationship in sucrose and how differential rearing impacts it.

Isolated rats had consistently lower aversive responding to both ethanol and quinine and other evidence indicates that environmentally induced differences in nociception may be involved. Ethanol directly activates the transient receptor potential vanilloid 1 (TRPV1), a heat-gated nociceptor also activated by spicy foods containing capsaicin (Trevisani et al. 2002), which induces an aversive/painful burning sensation in the mouth and the periphery. Mice reared in isolation have impaired TRPV1 nociception across multiple measures compared to group-reared controls (Horiguchi et al. 2013). Reduction in temperature-related pain sensitivity is also found in isolated rats (Petrovski et al. 2013). Some hypothesize that isolates have enhanced activation of descending inhibitory control over pain via serotonin (Horiguchi et al. 2013) while others suggest changes to the endogenous opioid system (Petrovski et al. 2013). When combined with the finding that somatosensory nociceptive (capsaicin) and bitter (quinine) gustatory signals overlap in the parabrachial nucleus (PbN; Li and Lemon 2019), the literature supports the possibility that the reduction of pain/aversive sensitivity to the sensation of ethanol and quinine may drive IC rats' reduced aversive responding to these substances.

A reduction of sensitivity to pain or aversive aspects of stimuli would also explain another discrepancy in previous literature: ethanol and sucrose are much more similar than ethanol and quinine in terms of gustatory neural responses (Di Lorenzo et al. 1986; Lemon et al. 2004; Lemon and Smith 2005). However, ethanol-paired conditioned taste aversion (CTA) generalizes to sucrose and quinine together, but not sucrose alone (Kiefer and Mahadevan 1993; Kiefer and Lawrence 1988; Kiefer et al. 1990; Di Lorenzo et al. 1986).

Electrophysiological recordings from gustatory neurons responding to a wide variety of

tastants in the nucleus of the solitary tract (NST) found that ethanol activates similar gustatory neurons to sucrose (Lemon et al. 2004; Di Lorenzo et al. 1986) and is more similar to sucrose than to quinine (Lemon and Smith 2005). However, ethanol-paired CTA did not generalize to sucrose solutions or quinine solutions (Kiefer et al. 1990; Kiefer and Lawrence 1988; Kiefer and Mahadevan 1993; Di Lorenzo et al. 1986) implying that the gustatory properties of sucrose alone and quinine alone are not sufficiently similar to ethanol to generalize. Thus, some component of quinine's gustatory properties is both shared with ethanol and essential for generalization of CTA but may not be observed in neurons of the gustatory region of the NST. This makes nociceptive activity, which overlaps with bitter gustatory signals in the PbN (Li and Lemon 2019) a good candidate for future studies interested in the source of sensation similarity between ethanol and quinine. Together, the evidence suggests the reduced aversive taste reactivity responding to quinine in IC rats may be mediated by similar mechanisms to reduced aversive taste reactivity responding to ethanol and are likely due to similarity between gustatory properties of ethanol and quinine.

Altogether, the evidence suggests isolated rats have a shift in allostatic set-point which may, in part, drive increased responding for a variety of rewards including ethanol and sucrose. Isolated rats' reduced aversion to ethanol's noxious aspects as a tastant are likely related to an altered experience of pain/discomfort such as increased pain tolerance. This aversion reduction might make the more aversive aspects of drugs of abuse, such as ethanol, less aversive to IC rats. Therefore, when the hedonic and aversive findings are taken together, IC rats may require more rewarding stimulation to reach the same subjective hedonic level compared to enriched rats while simultaneously being less responsive to the discomfort associated with bitter tastes or higher levels of rewarding stimulation. Conversely, enriched rats have enhanced liking of both sucrose and ethanol based on our taste reactivity results, but also have reduced wanting based on a larger number of operant experiments. This suggests that enrichment may offer a unique phenotype with divergent preferences for incentive motivation. In sum, our results provide further insight into the robust, complex, and lasting effects the early rearing environment can have on incentive motivation and substance use disorders.

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EC assigned IC and SC yoked	Water 1	Sucrose 0.1M	Sucrose 0.25M	Sucrose 0.5M	Quinine 0.5mM	Quinine 0.1mM	Ethanol 5%	Ethanol 10%	Ethanol 20%	Ethanol 30%	Ethanol 40%	Water 2
Day/Session:	1	2	3	4	5	6	7	8	9	10	11	12

Fig. 1.

A representative example of a substance presentation for an individual animal on an individual trial with members of the other groups yoked to the same presentation order. Please refer to the methods section for more details concerning the counterbalancing.

Fig. 2.
The interior of the taste reactivity chamber from the perspective of the video camera.

Fig. 3.

Mean $\pm$ S.E.M. **a** Total hedonic and **c** total aversive taste reactivity responses to ethanol (5–40% v/v) between enriched (EC), isolated (IC), and standard condition (SC) rats. Individual-level data and trend lines from the linear model for rats in each group are shown separate from mean data for ease of data illustration for both **b** total hedonic and **d** total aversive taste reactivity responses to ethanol. Hedonic responses (**a**, **b**): EC rats had significantly more hedonic responses to alcohol than IC rats (*** $p < .001$). ECs also had a significantly greater rate of decline in hedonic responses compared to SCs as ethanol concentration increased ($b_p = .005$). Aversive responses (**c**, **d**): As alcohol concentration increased, aversive responses increased (### $p < .001$). ECs had significantly greater aversive taste responses to ethanol compared to ICs ($*p < .05$).

Fig. 4.

Mean $\pm$ S.E.M. **a** Total hedonic and **c** total aversive taste reactivity responses to sucrose (0.1–0.5 M) between enriched (EC), isolated (IC), and standard condition (SC) rats.

Individual-level data and trend lines from the linear model for rats in each group are shown separate from mean data for ease of data illustration for both **b** total hedonic and **d** total aversive taste reactivity responses to sucrose. Hedonic responses (**a, b**): As sucrose concentration increased, hedonic responses increased ($^{##}p < .01$). ECs had significantly more hedonic responses to sucrose than ICs ($^{**}p < .01$). Aversive responses (**c, d**): Aversive responding to sucrose was low, no significant differences were observed.

Fig. 5.

Mean $\pm$ S.E.M. **a** Total hedonic and **c** total aversive taste reactivity responses to quinine (0.1–0.5 mM) between enriched (EC), isolated (IC), and standard condition (SC) rats. Individual-level data and trend lines from the linear model for rats in each group are shown separate from mean data for ease of data illustration for both **b** total hedonic and **d** total aversive taste reactivity responses to quinine. Hedonic responses (**a, b**): As quinine concentration increased, hedonic responses decreased ($^{\#}p < .05$). ECs had significantly more hedonic responses to quinine than ICs ($^{**}p < .01$). Aversive responses (**c, d**): As quinine concentration increased, aversive responses increased ($^{\#}p < .05$). ECs had significantly greater aversive taste responses to quinine compared to ICs ($^{**}p < .01$).

Fig. 6.

Mean $\pm$ S.E.M. **a** Total hedonic and **c** total aversive taste reactivity responses to water between enriched (EC), isolated (IC), and standard condition (SC) rats during the initial (water 1) and final (water 2) water sessions. Individual-level data for rats in each group and for each session are shown separate from mean data for ease of data illustration for both **b** total hedonic and **d** total aversive taste reactivity responses to water. Hedonic responses (**a**, **b**): ECs and SCs significantly increased hedonic responding from the initial to the final water session ($^{\dagger}p < .017$) and showed greater responding during the final water session compared to ICs ($*p < .017$). Aversive responses (**c**, **d**): Aversive responses increased from the initial to the final water session ($^{###}p < .001$).