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The Interaction of Ethanol Ingestion and Social Interaction with an Intoxicated Peer on the Odor-Mediated Response to the Drug in Adolescent Rats

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Abstract

Background—Using a social transmission of food preference paradigm in rats, we previously demonstrated that ethanol exposure during adolescence, as either an observer (interaction with an intoxicated conspecific) or demonstrator (intragastric infusion with ethanol), altered the reflexive odor-mediated responses to the drug. The two modes of exposure were equivalent in the magnitude of their effects. Human adolescents, however, are likely to experience the drug in a social setting as both an ethanol observer and demonstrator. That is, both interacting with an intoxicated peer and experiencing ethanol's post-ingestive consequences in conjunction with hematogenic olfaction. Therefore, we tested whether *combined* adolescent exposure as both an observer and demonstrator differed from either form of individual experience.

Methods—Beginning on postnatal day (P) 29, naïve rats received ethanol or water exposures in a social interaction paradigm as either an observer, a demonstrator or combined experience (where each animal in the interaction was, itself, an observer and demonstrator). Exposures occurred four times, once every 48 hours. On P37, the reflexive behavioral response to ethanol odor was tested, using whole-body plethysmography.

Results—The odor-mediated responses of adolescent ethanol observers, demonstrators and combined exposure animals all significantly differed from controls. Compared to controls, however, the magnitude of the behavioral effect was greatest in the combined exposure animals. Moreover, combined exposure as both an ethanol observer and demonstrator significantly differed from either form of individual ethanol experience.

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AUTHORS CONTRIBUTIONS

AME, LMY and SLY were involved in the design of the study, interpretation of the data, and preparation of the manuscript; LMY executed the study; AME and SLY conducted the data analysis; All authors read and approved the final manuscript. None of the authors having competing conflicts of interest.

Conclusions—Ethanol’s component chemosensory qualities are known to be central contributors to its acceptance and increases in the acceptability of ethanol’s odor, resulting from a social transmission experience, are predictive of enhanced ethanol avidity in adolescence. Our findings demonstrate that combined exposure as an observer and demonstrator, within a socially relevant framework, may represent a higher risk scenario for increased ethanol avidity in adolescence (and by extension adult persistence) as compared to the individual effects of direct ingestion or social experience with the drug.

Keywords

Ethanol Ingestion; Social Interaction; Adolescent Ethanol Exposure; Odor-Mediated Response to Ethanol; Adolescent Rat

INTRODUCTION

Alcohol use during adolescence is relatively common, with binge drinking being a particularly prominent method of exposure during teenage years (Johnston et al., 2012; Miller et al., 2007). Recent research has shown that approximately 7% of 8th graders, 15% of 10th graders and 22% of 12th graders reported consuming 5 or more drinks per occasion during a previous 2-week period (Johnston et al., 2012). The prominence of childhood and adolescent alcohol consumption is of noteworthy concern, not only because of the deleterious effects it has on the ongoing development of the nervous system (Spear, 2010), but also because of the relationship between early age drinking and the increased risk of developing an alcohol use disorder (AUD) later in life (DeWit et al., 2000; Grant and Dawson, 1997). Indeed, the National Longitudinal Alcohol Epidemiologic Survey showed that those individuals who reported an early age onset of drinking were more likely to meet the criteria for lifetime abuse and dependence (Grant and Dawson, 1997).

The number of factors influencing alcohol acceptance and intake are many, emerging differentially across both age and alcohol dose experienced (Spear, 2000). These factors include, but are not limited to: alcohol’s rewarding, social facilitating and anxiolytic effects, as well as its sedating, motor-impairing and aversive properties. With specific regard to alcohol’s aversive properties, the flavor profile of alcohol, which is comprised of the sensory integration of its smell, taste and oral irritating qualities, is considered to be an important contributing factor of its’ acceptance (Bachmanov et al., 2003). In this respect, the chemosensory processes fundamental to the flavor perception of alcohol have largely been viewed as static mechanisms that help to deter consumption of the drug (i.e., ethanol) because of its inherently unpalatable flavor attributes (aversive odor, bitter taste and oral irritation [e.g., Glendinning et al., 2012; Youngentob and Glendinning, 2009]). However, experience-induced neural and behavioral chemosensory plasticity throughout the lifespan (including fetal development) is a well-described mechanism (e.g., Hill, 2004, Hudson and Distel, 1998; Salcedo et al., 2005; Youngentob and Kent, 1995). Further, the available data demonstrates that animals gain survival benefits from highly plastic chemosensory processes that enhance an animal’s responsiveness to stimuli that are “deemed” salient (Hudson, 1999). This view notwithstanding, within the context of ethanol experience it has been argued that such plasticity (a developmentally adaptive process) works to the disadvantage

of the adolescent animal (e.g., Glendinning et al., 2012; Youngentob and Glendinning, 2009; Youngentob et al., 2007): enhancing the already inherent probability of initial intake (Spear, 2010) and continued consumption in adolescence (Hunt and Hallmark, 2001).

Alcohol consumption in adolescence often occurs in, and is influenced by, social situations (Bradizzi et al., 1999). The relevance of these social interactions to the potential for long-term abuse cannot be overstated. Varlinskaya and colleagues (Varlinskaya and Spear, 2004) demonstrated that adolescent animals were less sensitive to ethanol-induced social inhibition and more sensitive to its social facilitating properties. Moreover, they suggested that the facilitation of social interactions, which occurred during both drinking episodes and the post-ingestive recovery periods, was a mechanism for establishing persistent cycles of drinking in an already “at-risk” age group.

In consideration of the above, socially relevant experience with a novel odorant can alter the later consumption preference for the exposure stimulus. The social transmission of food preference model originally characterized by Galef and colleagues (Galef, 1981; Galef and Stein, 1985; Galef and Wigmore, 1983) centers on the finding that animals can obtain information regarding foods to consume based on an interaction with a peer that has recently ingested a diet novel to the conspecific: odor cues perceived on the breath of another animal (namely, the demonstrator) impact later food choices of the conspecific (namely, the observer). This basic phenomenon has been demonstrated to influence ethanol acceptability. Several studies have shown, for example, that both infant and adolescent observer rats with no prior ethanol experience will show enhanced drug consumption as a consequence of interaction with a peer that was administered ethanol (Fernandez-Vidal and Molina, 2004; Hunt and Hallmark, 2001; Hunt et al., 2001). These increases in intake appear to be driven, at least in part, by altering the odor attributes of ethanol (an important component of its overall flavor profile) for the observer animals. For example, Eade and colleagues demonstrated that ethanol odor exposure within a social context yielded enhanced odor-mediated responses to ethanol in adolescent observer animals (Eade and Youngentob, 2009; Eade et al., 2009). Moreover, in extension to this observation, the pharmacologic properties of ethanol, in conjunction with, perhaps, hematogenic olfaction (Maruniak et al., 1983) also appeared to influence the odor-mediated responses of the intoxicated demonstrator animals. Like observer animals, demonstrators having experienced ethanol via intragastric infusion in adolescence showed an enhanced behavioral response to its odor (Eade and Youngentob, 2009).

Given the propensity for adolescent alcohol consumption to occur in, and be influenced by, social interactions (Bradizzi et al., 1999; Varlinskaya and Spear, 2004), it is likely that human adolescents will experience the alcohol odor-related consequences of being both an observer and demonstrator. Therefore, as outlined below, we modified the traditional social transmission paradigm such that adolescent animals received either a single (i.e., they were an observer or demonstrator) or combined (i.e., they were both an observer and demonstrator) mode of ethanol or water control exposure in adolescence. Using this approach, we tested the hypothesis that the enhanced odor-mediated response of combined ethanol exposure, as both an observer and demonstrator would be greater than either form of individual ethanol experience alone.

MATERIALS AND METHODS

Ethics Statement

Upstate Medical University's Institutional Animal Care and Use Committee approved all experimental methods. Processes were in place to reduce discomfort where applicable.

Generation of Subjects

The present study utilized 96 naive Long-Evans Hooded rats. These animals were the offspring of 12 dams.

Pregnant Long-Evans female rats (Harlan-Sprague Dawley, Indianapolis, IN) were housed in a temperature and humidity controlled environment with a fixed 12-hour light/dark cycle. Dams were provided *ad libitum* access to both food and water throughout gestation. Litters were sexed and reduced to ten pups (when applicable) on postnatal (P) day 2, with the constraint that any given litter had no less than four males and four females. Absent that gender balance, the litter was not utilized.

We generated four different types of adolescent experimental exposure groups using a modification of the social transmission of food preference paradigm originally described by Galef (Galef, 1981; Galef and Stein, 1985; Galef and Wigmore, 1983). More specifically, using same-sex pairs of male and female siblings, we generated animals that received ethanol (E) experience as, only an ethanol observer (OE), only an ethanol demonstrator (DE) or combined exposure in which *both* animals in a pair served as both an ethanol observer and ethanol demonstrator (CE). Moreover, we generated a combined exposure water group, as the appropriate control for all conditions, in which both animals served as a water observer and demonstrator (CW) (*see below for details*).

Under ideal conditions, a single dam would produce all of the pups needed to generate a single experimental testing "block" in which all exposure conditions were represented. However, in order to provide one animal, per sex, from each of the 4 treatment groups, would have required each dam to produce 6 males and 6 females (12 animals). Since this was an unrealistic expectation, we used triads of litters to produce the pairs of littermates needed for this study, as follows. The litters derived from triads of weight-matched dams were randomly assigned to one of three different adolescent exposure strategies (Fig. 1). Within a litter specific strategy only two of three general exposure conditions were represented (i.e., combined ethanol exposure as both an observer and demonstrator, single mode of ethanol exposure [as either an observer or demonstrator] or combined water control). To generate the experimental animals for this study (8male and 8 female subjects for each of the OE, DE, CE and CW exposure groups) required four triads of dams. Note that, by virtue of the required design, combined ethanol and combined water control pairs produced twice as many animals as the individual ethanol pairs. Therefore, only one animal from each combined exposure pair was randomly selected to participate in subsequent behavioral testing.

Adolescent Exposures

Within a particular litter specific exposure strategy, at weaning (P21), eight pups (4 male; 4 female) were separated into 4 same-sex pairs (Eade et al., 2009; Eade and Youngentob, 2009) that were then randomly allocated amongst the 2 treatment conditions assigned to the litter. Once an animal was assigned to a treatment condition it remained in that experimental group throughout the study.

For individual ethanol exposure pairs (strategies 1 and 3), one animal was randomly assigned as the OE or DE. DE animals received ethanol exposure through intragastric (i.g.) infusion of 1.5g/kg (e.g., Eade et al., 2009; Fernandez-Vidal and Molina, 2004; Hunt et al., 2001) of ethanol (12.6% v/v solution made from 100% ASC/USP food grade ethanol). The OE animals received an i.g. infusion of an equivalent volume of tap water followed by social interaction with their intoxicated peer, as outlined below. Note that, by virtue of this approach, OE animals were both a water demonstrator and ethanol observer within their social interaction pair, whereas, the DE animals were both a water observer and ethanol demonstrator. By contrast to the above, both animals received ethanol infusion within a conspecific pair in the CE group (strategies 1 and 2) where each rat served as both an ethanol observer and demonstrator in the subsequent social interaction. The observer/demonstrator pairs in the CW group both received a water infusion dose prior to social interaction. Thus, in this study, all animals received some form of i.g. infusion (either ethanol or water), thereby controlling for any potential stress effect of the infusion procedure, itself (Eade and Youngentob, 2009).

Adolescent experience through social interaction of same-sex pairs occurred on 4 separate occasions: P29, P31, P33, and P35, respectively. On each exposure day, the pairs were first separated for 1 hour prior to the designated social interaction time by randomly removing one littermate from the home cage with the restriction that each animal in a pair was removed on two of the four occasions. Thirty minutes into the separation period, animals within a pair received their appropriate i.g. infusion. Animals remained separated for 30 minutes following infusion. This time period allowed for the ethanol to be absorbed and then expired through the respiratory system in the animals receiving drug infusion (e.g., Molina and Chotro, 1989; Pohorecky and Brick, 1982). At the end of this separation period, animals were returned to their home cages for 30 minutes of social interaction. Following the social interaction, animals were again separated for 4 hours (the animal initially removed was removed again). This period of time permitted the ethanol infused animals' to clear the drug from their systems prior to being re-housed with their sibling overnight (e.g., Fernandez-Vidal and Molina, 2004).

Olfactory-Mediated Response to Ethanol

For each experimental animal, the reflexive odor-mediated response to ethanol was tested once on P37, 48 hours after the final social interaction. Testing was accomplished using a previously established unbiased method for the monitoring and quantification of an animal's responsiveness to odorant stimuli (e.g., Eade and Youngentob, 2009; Eade and Youngentob, 2010; Eade et al., 2009; Youngentob, 2005; Youngentob et al., 2007; Youngentob and Glendinning, 2009). The detailed specifics of the procedure have been previously described

(*ibid*). Briefly, an untrained, freely moving rat was placed in a Plexiglas cylindrical testing chamber that permitted the fast onset and clean out of odorant stimuli. Stimuli were delivered into the chamber for six seconds with a continuous airflow, using a flow-dilution olfactometer. Whole-body plethysmography was used to monitor the inherent stimulus-induced sniffing responses (changes in airflow patterns of respiration) following the delivery of air or different concentrations of the odorant ethanol. The behavioral testing parameters, generation of stimuli, and the gathering of behavioral data was computer controlled (*ibid*).

Following 40 air-only habituation trials, each animal was tested with 5 blocks of the randomized presentation of 10 air and 10 ethanol odor stimuli per block of 20 trials. Within each block, only one of 5 different concentrations of ethanol odorant (0.313%, 0.625%, 1.25%, 2.5% and 5% of vapor saturation at 20°C) was presented and the different concentrations were tested from low to high.

Data Analysis

Following an established analytic approach (e.g., Eade and Youngentob, 2009; Mantella et al., 2013; Youngentob and Glendinning, 2009; Youngentob et al., 2012), the recorded changes in airflow resulting from the animals' sniffing responses to ethanol odor were deconstructed into fourteen response measures. These measures were: respiratory frequency; number of inspiratory and expiratory sniffs; average duration, volume, flow rate and peak flow rate of inspiratory and expiratory sniffs; total inspiratory and total expiratory volumes; and the total apneic duration. In this regard, the multivariate data did not allow an explicit test of experimental main effects. Moreover, previous work has shown that examining an individual variable was insufficient to evaluate the complex behavioral responses to odorant. Therefore, as outlined below, the values for each sniffing characteristic were used to establish a single principal measure or "behavioral index" that represented the animals' odor-mediated behavioral responses to ethanol odor. This index, in turn, was used to test the main effects of treatment (*ibid*).

Based on the testing paradigm described above, each animal initially contributed a 14 x 5 response matrix (fourteen measures of reflexive sniffing responses for each of the five concentrations of ethanol odor) to the total data. In order to reduce the high dimensionality of the data, we used principal components analysis (PCA) to first reduce the fourteen measures, within an *a priori* set of data related to specific hypotheses, to *N* orthogonal response vectors (namely, Factors of the PCA analyses). In other words, the statistical transformation converted the fourteen correlated sniffing measures into a set of fourteen compressed values of linearly uncorrelated variables (i.e., Factors) each which contained all response measure. These Factors, in turn, allowed us to evaluate how the data varied collectively based on variance. Note that, for each *a priori* hypothesis to be evaluated there was a particular principal effect to be examined such that what was relevant to evaluate one *a priori* hypothesis was not most relevant to test another (*ibid*). In short, the PCA analysis reduced each of the animals' individual 14 x 5 response matrix to an *N* x 5 data matrix (*N* PCA Factor values for each concentration).

To create a behavioral Index for each rat that incorporated the behavioral responses across all concentrations of ethanol odorant tested, multivariate linear regression analyses were

performed on each PCA factor. For each regression analysis the five behavioral response values (one at each concentration) served as the dependent variables and postnatal treatment as the independent variable. The regression results for each PCA Factor provided estimates of the coefficients for each concentration of odorant plus a constant. The behavioral “Index” value calculated from each PCA Factor for an individual animal was the sum of the constant from the regression analysis, plus the corresponding PCA value at each concentration of ethanol odor multiplied by the estimated coefficient. This approach resulted in a set of coordinates that located each animal in an N -dimensional behavioral response space. Moreover, the N -dimensional response data were used in later analyses of specific main effects.

RESULTS

The principal goal of this study was to determine the extent to which different types of adolescent experience with either the pharmacologic consequences of ethanol and/or ethanol odor through social interaction with an intoxicated peer altered the reflexive odor-mediated behavioral response to the drug. As such, we tested the null hypothesis that there was no difference in the behavioral response to ethanol odor among the CE, OE, DE and CW exposure groups. The initial test of overall differences related to main effects involved the evaluation of a 3-dimensional (D) solution (from the PCA) that accounted for 98.24% of the total variance. In determining three dimensions as the appropriate solution, the objective in this study was to maximize our potential to accurately map the treatment group differences in a behavioral response space while maintaining sufficient error degrees of freedom for the formal analysis of our overall hypothesis (Youngentob et al., 2001). In this regard, because of the large number of treatment groups, lower dimensional solutions appeared to obscure an accurate representation of the relational differences between the different modes of adolescent exposure, whereas adding additional dimensions (i.e., PCA Factors) did not capture further meaningful dissimilarities or variance. The relational data illustrated in Figure 2 shows the position of the four treatment groups in a 3D ethanol odor-mediated behavioral space. The behavioral space is the 3D composite behavioral index solution, based on the resultant 3 PCA factors, with the average ($\pm$ 3D s.e.m.) of each of the adolescent exposure groups representing points in the space. In this space, the magnitude to which the rats' inborn responses to ethanol odor in each group were alike plots as the proximity of their locations in the 3D graph (e.g., Youngentob and Glendinning, 2009; Youngentob et al., 2012). As can qualitatively be seen in this figure, there is a clear boundary between the CE versus OE and DE animals, the CE versus CW animals, and the CW versus OE and DE rats. Multivariate analysis of covariance (Wilk's Lambda criterion) provided evidence for an overall altered behavioral response to ethanol odor as a function of adolescent mode of exposure ($F [9,129] = 2.33, P = 0.02$). On average, there was no strong evidence for an overall effect of sex ($F [3,53] = 2.37, P = 0.08$) or sex by treatment interaction ($F [9,129] = 0.45, P = 0.9$).

To give broader interpretation to the above overall result, we tested an additional set of *a priori* hypotheses (based on adjusted error terms) to explore further the consequence of different modes of adolescent exposure on the reflexive behavioral response to ethanol odor. First, relative to our prior observations, using a traditional social transmission paradigm (i.e.,

a differential enhanced odor-mediated response between observers and demonstrators, in the face of comparable net differences relative to their control counterparts [Eade and Youngentob, 2009]), we tested whether the behavioral responses of adolescent OE and DE animals again differed both from each other and from a uniform CW control group. Recall that, by contrast to the standard paradigm, in which only demonstrator animals (ethanol or water) receive i.g. infusion, in the current study all animals experienced the procedure (either ethanol [DE and CE animals] or tap water [OE and CW animals] to control for the potential stress effect of the method, itself, across all animals.

The relational data illustrated in Figure 3 (Panel A) shows the position of the OE, DE and CW animals in a 2-dimensional (2D) ethanol odor-mediated behavioral space. The behavioral space is the 2D composite behavioral index solution, based on a resultant 2 PCA Factors, with the average ($\pm$ 2D s.e.m.) of each of the three adolescent exposure groups representing points in the space. As outlined below, the use of the 2D solution permitted us to evaluate paired group comparisons that otherwise could not be accomplished with higher dimensionality. Consistent with the qualitative relationship illustrated in Figure 2, Figure 3 (Panel A) highlights that the OE and DE animals have essentially an identical odor-mediated response to ethanol whereas both groups appear distinctly different from the CW controls. To formally evaluate these subsidiary hypotheses, single variable significance tests (two-tailed t : $P < 0.05$) were done on the joint weighted city-block distance of the effect sizes for the two indexes related to two adolescent exposure groups (Mantella et al., 2013; Youngentob et al., 2012). The weighted distance was the sum of the absolute values of the individual effect sizes for each index. As previously described, the weighting scheme was based on the excess of each principal component factor's Eigen value above the Kaiser criterion of 1 (*ibid*). As seen in Figure 3 (Panel B), the effect size comparisons between the three adolescent treatment conditions confirmed our expectation that, on average, whereas both DE and OE groups were significantly different from CW controls (t [45] = 2.22, $P = 0.03$ and t [45] = 3.0, $P = 0.005$, respectively) the OE and DE groups were not different from each other (t [45] = 0.78, $P > 0.45$). The equivalency in the response of the OE and DE animals stands in stark contrast to our prior result, noted above (Eade and Youngentob, 2009), and further highlights that i.g. infusion is a contributing factor in the animals' behavioral response to odor.

To interpret further the general finding of an overall consequence of adolescent treatment, it was also necessary to consider: (1) whether CE exposure, indeed, differed from the OE and DE animals; and (2) if so, whether, relative to CW controls, CE exposure, resulted in an altered odor-mediated ethanol response. In regard to question 1, given the stark equivalency between the OE and DE animals, we based our analysis of CE versus the individual modes of exposure on a joint OE and DE effect. This approach permitted a more tightly focused test of our *a priori* hypothesis while at the same time preserving any nominal average effect, if any, of both OE and DE exposures (Mantella et al., 2013). Both the overall 3D representation of the four treatment groups (Fig. 2) as well as the hypothesis specific 2D plot shown in Figure 4 (Panel A) provided qualitative evidence for a differential effect between CE and the joint individual types of adolescent ethanol exposure. Formal analysis of the ethanol odor-mediated effect size (in 2 dimensions) showed a highly significant overall difference (t [45] = 3.17, $P = 0.003$) (Fig. 4; Panel B).

Figure 5 (Panel A) illustrates the relative position of the CE and CW animals in an ethanol odor stimulus-induced behavioral space. As can be seen, there is a clear demarcation between the two groups in both dimensions of the 2D plot. The formal evaluation of the magnitude of the behavioral alteration (Fig. 5; Panel B) demonstrated that relative to CW controls, on average, there was an overall significant effect of CE exposure on the reflexive odor-mediated response to ethanol ($t[30] = 2.51$; $P = 0.02$).

DISCUSSION

The age range of adolescence is a developmental epoch when interaction with peers plays a particularly important role in shaping later behaviors. Alcohol is a readily available and commonly used drug among this age group and socio-environmental context plays prominently in intake behavior. The available data demonstrate the high probability for alcohol consumption to occur in conjunction with and be shaped by social interactions (e.g., Bradizzi, 1999). Of particular note, studies on adolescent alcohol consumption have revealed that peers' drinking is one of the strongest correlates of use, especially for young adolescents (Kelly, 2012). Moreover, there is substantial evidence that peer alcohol use within a social context is associated with both the initiation and development of long-term use and abuse (e.g., Ali & Dwyer, 2009; Ali & Dwyer, 2010).

Increased levels of alcohol consumption in a social setting is not restricted to human adolescents. Using a rat model, several studies have demonstrated the important role of social peer interactions in adolescent intake (e.g., Doremus et al., 2005, Varlinskaya et al., 2015). Such studies have, for example, focused on age and sex differences, as well as the personality factors that can impact long-term use and abuse (namely, to enhance positive feelings [e.g., euphoria, relaxation] versus ethanol's anxiolytic effects [attenuate negative feelings, e.g., stress and tension]). These contributors to ethanol acceptance notwithstanding, other work has examined the role that social interactions specifically play in influencing future ethanol preference behaviors. Animals learn through social interaction what flavors (the integration of a food substance's smell, taste and oral irritation) to accept, and ethanol flavor perception is known to be an important determinant of consumption (Bachmanov et al. 2003). In this respect, the olfactory system, and its contribution to ethanol acceptance has historically been considered a static sensory mechanism that functions as an inhibitor of intake by virtue of its inherently aversive odor. However, naïve adolescent rats (observer animals in the social transmission paradigm [e.g., Galef, 1981, Galef and Stein, 1985, Galef and Wigmore, 1983]) will increase their preference for ethanol odor following exposure to an intoxicated peer (demonstrator animals that received direct ethanol exposure) in a social setting, as well as display enhanced ethanol intake as a consequence of that interaction [e.g., Eade et al., 2009, Hunt et al., 2001, Fernandez-Vidal et al., 2004, Maldonado et al., 2008]. Thus, the animal literature suggests that social interactions, among other things, heightens the preference for ethanol consumption, at least in part, by way of a chemosensory mechanism.

While human adolescents can, in general, experience alcohol directly, through personal ingestion, and indirectly, through interacting with peers who have consumed the drug, they are most likely to experience both modes of exposure simultaneously. With regard to the first

two exposure possibilities, animal models have shown that adolescent demonstrators, like observers, manifest an enhanced behavioral response to ethanol odor (Eade and Youngentob, 2009). However, there was a marked differential enhanced odor response between observers and demonstrators, despite similar net enhancements, relative to controls. This observation suggested the presence of a stress effect from the intra-gastric infusion technique used to expose demonstrator animals to ethanol in the social transmission paradigm (*ibid*).

The present study expands upon this prior work in two fundamental ways. First, using a single overarching design which, (1) incorporated all possible combinations of direct and indirect exposures, and (2) a common control group, we determined the degree to which adolescent experience with either the post-ingestive consequences of ethanol and/or ethanol odor through interactions with an intoxicated peer altered the reflexive behavioral response to ethanol odor. Importantly, we also controlled for the potential confounding effect of intra-gastric infusion across the different experimental groups, such that all animals received some form of infusion (either ethanol or water).

In keeping with previous findings (Eade and Youngentob, 2009), compared to controls, both ethanol observer (OE) and demonstrator (DE) animals manifested an altered odor-mediated response to ethanol (Figs. 2 and 3). However, by contrast to our prior study, we found no evidence for a differential odor-mediated response between the two single modes of exposure animals. The absence of a differential result between the OE and DE animals in the present study confirmed our expectation of an untoward effect of intra-gastric infusion and that the procedure, itself, confounds the effects of both forms of ethanol-related experience. Moreover, it would appear from these results that both forms of exposure represent equivalent risk factors for altering ethanol flavor perception. The data also suggest it likely that the infusion procedure devalued any experienced-induced alterations in the response to ethanol odor. In this respect, adolescents are known to be more sensitive to stressors (Spear, 2000) and such exposures have been shown to impact adolescent responses to ethanol, for example, decreasing drug intake (Brunell and Spear, 2005).

A key observation of the present study was the finding that combined exposure as both an observer + demonstrator (CE) was different than either mode of exposure alone (OE and DE), as well as from the combined water controls (CW) (Figs. 2 & 4). The exact relative magnitude, in 3D, of the various exposure groups reflexive responses is a matter of conjecture at this time. Nonetheless, a consideration of how OE, DE and CE exposures mechanistically differ and how such differences would influence the experience-induced enhancement can provide insight into this question.

Several lines of evidence clearly demonstrate the olfactory system is developmentally plastic into adulthood (e.g., Salcedo et al, 2005; Youngentob and Kent, 1995). Rodents that received salient odor experience via both operant (*ibid*) or classical conditioning paradigms (e.g., Coopersmith and Leon, 1984,1986) display, at a physiologic level, tuned or enhanced epithelial and olfactory bulb maps to the exposure odorant. These maps, in turn, form the basis for the encoding of odorant quality (Kent et al., 1995; Youngentob et al., 2006). The data also show the olfactory bulb encodes the associative significance of an odorant (e.g., Wilson and Sullivan, 1990). Thus, salient odor exposure as either an observer or

demonstrator would, individually, be expected to enhance both the fundamental encoding of ethanol odor, as well as its associative relevance. In observer (OE) animals, this process would occur through the pairing of ethanol odor with social peer interactions and such interactions are known to result in enhanced ethanol avidity (e.g., Hunt and Hallmark, 2001; Hunt et al., 2001). Indeed, the social interaction is critical for the associative effect (March et al., 2013). For the demonstrator (DE) animals, both the pharmacologic properties of ethanol paired together with hematogenic olfaction (e.g., VanDishoeck HAE, Versteeg N, 1957; Maruniak et al., 1983) would also influence these same processes and the results of the present study suggest the magnitude of the OE and DE effects were similar. So what of combined exposures (CE)? Given the foregoing mechanisms, and the prevailing data on the social transmission of food preferences, our present results are consistent with the proposition of an augmentation effect and that the behavioral responsiveness to ethanol odor stemming from combined exposures likely represents a critical component in developing progressive patterns of ethanol use and abuse in adolescence. Indeed, using another form of combined exposure, i.e., the combination of prenatal and adolescent exposures, we have shown an augmented response to ethanol odor relative to either form of individual odorant exposure alone (Eade et al., 2009; Eade and Youngentob, 2010). Moreover, the interaction of pre- and postnatal exposure can yield enhanced ethanol avidity relative to the effect of either exposure alone (e.g., Chotro et al., 1996; Honey and Galef, 2003).

Conclusion

The chemosensory qualities of ethanol are central contributors to its acceptance and increases in ethanol odor preference, resulting from a social transmission experience, are predictive of increased avidity in adolescence. Our findings demonstrate that combined exposure as an observer and demonstrator, within a socially relevant framework, may represent a higher risk scenario for increased ethanol intake in adolescence (and by extension adult persistence) as compared to the individual effects of direct ingestion or social experience with the drug, alone.

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References

- Ali MM, Dwyer DS. Estimating peer effects in adolescent smoking behavior: A longitudinal analysis. *J Adol Health*. 2009; 45:402–408.
- Ali MM, Dwyer DS. Social network effects in alcohol consumption among adolescents. *Addict Behav*. 2010; 35:337–342. [PubMed: 20051311]
- Bachmanov AA, Kiefer SW, Molina JC, Tordorff MG, Duffy VB, Bartoshuk LM, Mennella JA. Chemosensory factors influencing alcohol perception, preferences, and consumption. *Alcohol Clin Exp Res*. 2003; 27:220–31. [PubMed: 12605071]
- Bradizzi CM, Reifman A, Barnes GM. Social and coping reasons for drinking: predicting alcohol misuse in adolescents. *J Stud Alcohol*. 1999; 60:491–499. [PubMed: 10463805]
- Brunell SC, Spear LP. Effect of stress on the voluntary intake of a sweetened ethanol solution in pair-housed adolescent and adult rats. *Alcohol Clin Exp Res*. 2005; 29:1641–1653. [PubMed: 16205364]

- Chotro MG, Kraebel KS, McKinzie DL, Molina JC, Spear N. Prenatal and postnatal ethanol exposure influences preweanling rats' behavioral and autonomic responding to ethanol odor. *Alcohol*. 1996; 13:377–385. [PubMed: 8836327]
- Coopersmith R, Leon M. Enhanced neural response to familiar olfactory cues. *Science*. 1984; 225:849–851. [PubMed: 6474157]
- Coopersmith R, Leon M. Enhanced neural response by adult rats to odors experienced early in life. *Brain Res*. 1986; 371:400–403. [PubMed: 3697770]
- DeWit DJ, Adlaf EM, Offord DR, Ognorne AC. Age at first alcohol use: a risk factor for the development of alcohol disorders. *Am J Psychiatry*. 2000; 157:745–750. [PubMed: 10784467]
- Doremus TL, Brunell SC, Rajendran P, Spear LP. Factors influencing elevated ethanol consumption in adolescent relative to adult rats. *Alcohol Clin Exp Res*. 2005; 29:1796–1808. [PubMed: 16269909]
- Eade AM, Sheehe PR, Molina JC, Spear NE, Youngentob LM, Youngentob SL. The consequence of fetal ethanol exposure and adolescent odor re-exposure on the response to ethanol odor in adolescent and adult rats. *Behav Brain Funct*. 2009; 5:3. [PubMed: 19146665]
- Eade AM, Youngentob SL. Adolescent ethanol experience alters immediate and long-term behavioral responses to ethanol odor in observer and demonstrator rats. *Behav Brain Funct*. 2009; 5:23. [PubMed: 19497110]
- Eade AM, Youngentob SL. The interaction of gestational and postnatal ethanol experience on the adolescent and adult odor-mediated responses to ethanol in observer and demonstrator rats. *Alcohol Clin Exp Res*. 2010; 34:1705–13. [PubMed: 20608909]
- Fernandez-Vidal JM, Molina JC. Socially mediated alcohol preferences in adolescent rats following interactions with an intoxicated peer. *Pharmacol Biochem Behav*. 2004; 79:229–41. [PubMed: 15501298]
- Galef, BG, Jr. Development of flavor preferences in man and animals: the role of social and nonsocial factors. In: Aslin, RN.; Alberts, JR.; Petersen, MR., editors. *Development of Perception*. Volume 4: Psychobiological Perspectives. Academic; New York: 1981. p. 411–431.
- Galef BG, Stein M. Demonstrator influence on observer diet preference: analysis of critical social interactions and olfactory signals. *Animal Learn Behav*. 1985:13.
- Galef BG Jr, Wigmore SW. Transfer of information concerning distant foods: A laboratory investigation of the 'information-centre' hypothesis. *Anim Behav*. 1983; 31:748–758.
- Glendinning JJ, Simons YM, Youngentob L, Youngentob SL. Fetal ethanol exposure attenuates aversive oral effects of TrpV1, but not TrpA1 agonists in rats. *Experimental Biology and Medicine*. 2012; 237:236–40. [PubMed: 22378825]
- Grant BF, Dawson DA. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse*. 1997; 9:103–110. [PubMed: 9494942]
- Honey PL, Galef BG Jr. Ethanol consumption by rat dams during gestation, lactation and weaning increases ethanol consumption by their adolescent young. *Dev Psychobiol*. 2003; 42:252–260. [PubMed: 12621651]
- Hunt PS, Hallmark RA. Increases in ethanol ingestion by young rats following interaction with intoxicated siblings: a review. [Review]. *Integrative Physiological & Behavioral Science*. 2001; 36:239–248. [PubMed: 11777018]
- Hunt PS, Holloway JL, Scordalakes EM. Social interaction with an intoxicated sibling can result in increased intake of ethanol by periadolescent rats. *Dev Psychobiol*. 2001; 38:101–109. [PubMed: 11223802]
- Johnston, LD.; O'Malley, PM.; Bachman, JG.; Schulenberg, JE. *Monitoring the Future national results on adolescent drug use: Overview of key findings, 2011*. The University of Michigan; Ann Arbor: Institute for Social Research; 2012.
- Kelly AB, Chan GCK, Toumbourou JW, O'Flaherty M, Homel R, Patton GC, Williams J. Very young adolescents and alcohol: evidence of a unique susceptibility to peer alcohol use. *Addict Behav*. 2012; 37:414–419. [PubMed: 22217919]
- Kent PF, Youngentob SL, Sheehe PR. Odorant-specific spatial patterns in mucosal activity predict perceptual differences among odorants. *J Neurophysiol*. 1995; 74:1777–1781. [PubMed: 8989412]

- Maldonado AM, Finkbeiner LM, Kirstein CL. Social interaction and partner familiarity differentially alter voluntary ethanol intake in adolescent male and female rats. *Alcohol*. 2008; 42:641–648. [PubMed: 18922665]
- Mantella NM, Kent PF, Youngentob SL. Fetal nicotine exposure increases preference for nicotine odor in early postnatal and adolescent, but not adult, rats. *PLoS One*. 2013; 8:e84989. [PubMed: 24358374]
- March SM, Pautassi RM, Nizhnikov M, Fernandez-Vidal J, Spear NE, Molina JC. Olfactory preference for ethanol following social interaction with an intoxicated peer in adolescent rats exposed to ethanol in-utero. *Psicothema*. 2013; 25:355–362. [PubMed: 23910751]
- Maruniak JA, Silver WL, Moulton DG. Olfactory receptors respond to bloodborne odorants. *Brain Res*. 1983; 265:312–316. [PubMed: 6850336]
- Miller JW, Naimi TS, Brewer RD, Jones SE. Binge drinking and associated health risk behaviors among high school students. *Pediatrics*. 2007; 119:76–85. [PubMed: 17200273]
- Molina JC, Chotro MG. Acute alcohol intoxication paired with appetitive reinforcement: effects upon ethanol intake in infant rats. *Behav Neural Biol*. 1989; 51:326–45. [PubMed: 2730497]
- Pohorecky LA, Brick J. A new method for the determination of blood ethanol levels in rodents. *Pharmacol, Biochem, Behav*. 1982; 16:693–696. [PubMed: 7089027]
- Pomery EA, Gibbons FX, Reis-Bergan M, Gerrard M. From willingness to intention: experience moderates the shift from reactive to reasoned behavior. *Pers Soc Psychol Bull*. 2009; 35:894–908. [PubMed: 19429884]
- Salcedo E, Zhang C, Kronberg E, RESTREPO D. Analysis of training-induced changes in ethyl acetate odor maps using a new computational tool to map the glomerular layer of the olfactory bulb. *Chem Senses*. 2005; 30:615–26. [PubMed: 16141292]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev*. 2000; 24:417–463. [PubMed: 10817843]
- Spear, LP. *The Behavioral Neuroscience of Adolescence*. New York: WW Norton and Company; 2010.
- Varlinskaya EI, Spear LP. Acute ethanol withdrawal (hangover) and social behavior in adolescent and adult male and female Sprague-Dawley rats. *Alcohol Clin Exp Res*. 2004; 28:40–50. [PubMed: 14745301]
- Varlinskaya EI, Truxell EM, Spear LP. Ethanol intake under social circumstances or alone in Sprague-Dawley rats: Impact of age, sex, social activity, and social anxiety-like behavior. *Alcohol Clin Exp Res*. 2015; 39:117–125. [PubMed: 25623411]
- VanDishoeck HAE, Versteeg N. On the problem of hematogenic olfaction. *Arch Otolaryngol*. 1957; 47:396–401.
- Wilson DA, Sullivan RM. Olfactory associative conditioning in infant rats with brain stimulation as reward. I. Neurobehavioral consequences. *Dev Brain Res*. 1990; 53:215–221. [PubMed: 2357795]
- Youngentob SL. A method for the rapid automated assessment of olfactory function. *Chem Senses*. 2005; 30:219–229. [PubMed: 15741600]
- Youngentob SL, Glendinning JI. Fetal ethanol exposure increases ethanol intake by making it smell and taste better. *Proc Natl Acad Sci USA*. 2009; 106:5359–5364. [PubMed: 19273846]
- Youngentob SL, Kent PF. Enhancement of odorant-induced mucosal activity patterns in rats trained on an odorant identification task. *Brain Res*. 1995; 670:82–88. [PubMed: 7719728]
- Youngentob SL, Johnson BA, Leon M, Sheehe PR, Kent PF. Predicting Odorant quality perceptions from multidimensional scaling of olfactory bulb glomerular activity patterns. *Behav Neurosci*. 2006; 120:1337–1345. [PubMed: 17201479]
- Youngentob SL, Molina JC, Spear NE, Youngentob LM. The effect of gestational ethanol exposure on voluntary intake in early postnatal and adult rats. *Behav Neurosci*. 2007; 121:1306–1315. [PubMed: 18085883]
- Youngentob SL, Kent PF, Youngentob LM. Gestational naltrexone ameliorates fetal ethanol exposures enhancing effect on the postnatal behavioral and neural response to ethanol. *Exp Biol Med*. 2012; 237:1197–1208.
- Youngentob SL, Margolis FL, Youngentob LM. OMP gene deletion results in an alteration in odorant quality perception. *Behav Neurosci*. 2001; 115:626–631. [PubMed: 11439451]

Figure 1.

Three different adolescent ethanol exposure strategies were used. Triads of age-matched litters of pups were randomly allocated to strategies 1, 2, or 3. Within each litter, a total of 8 animals (4 per sex) were separated into 4 same-sex pairs that were then further randomly assigned to the 2 treatment exposure conditions within an individual strategy. CE = combined ethanol exposure in which both animals in the pair served as both an ethanol observer (exposure through social interaction with an intoxicated peer) and ethanol demonstrator (exposure through intragastric infusion with ethanol); OE = an ethanol observer through social interaction; DE = an ethanol demonstrator through intragastric infusion; CW = combined water exposure in which both animals in the pair served as both a water observer (exposure through social interaction with a tap water infused peer) and water demonstrator (exposure through intragastric infusion with tap water). Note that, OE animals received an intragastric infusion with tap water. See text for further details.

Figure 2.

The relative position of the CE, OE, DE and CW exposure groups in a 3-dimensional (3D) odor-mediated behavioral response space. For any two adolescent exposure groups, the extent to which their responses to ethanol odor were similar maps as the degree of closeness in the 3D space. The data are the adjusted least square treatment means ($\pm$ 3D s.e.m.) from the MANCOVA (see text for details).

Figure 3.

The reflexive odor-mediated behavioral response to ethanol varies as a consequence of adolescent OE, DE and CW exposure. Panel A shows the relative location of the three adolescent exposure groups in a two-dimensional (2D) ethanol odor-mediated behavioral response space. The data points are the adjusted least square mean sniffing indexes (± 2 D s.e.m.) as a function of the adolescent exposure groups (Solid circles = OE exposed; Solid squares = DE exposed; solid triangle = CW exposed). Panel B shows the ethanol odor-mediated weighted effect size comparisons. The effect sizes (mean $\pm$ s.e.m.) were calculated from the data illustrated in Panel A.

Figure 4.

The reflexive odor-mediated behavioral response to ethanol differs for the CE vs. combined OE and DE exposure groups. In Panel A, the values represent the mean ($\pm$ two-dimensional s.e.m.) relative position of CE versus combined individual exposure groups in a stimulus-induced behavioral response space (Solid circle = CE; Solid Squares = Joint OE & DE exposures). Panel B shows the ethanol odor-mediated weighted effect size (mean $\pm$ s.e.m.) calculated from the data illustrated in Panel A.

Figure 5.

CE and CW animals differ in their reflexive odor-mediated behavioral response to ethanol. Panel A illustrates the relative location of the CE and CW animals in an ethanol odor-mediated behavioral space. The data points are the adjusted least square mean sniffing indexes ($\pm$ two-dimensional s.e.m.) as a function of exposure groups (Solid circle = CE; Solid Squares = CW). Panel B illustrates the ethanol response-mediated weighted effect size (mean $\pm$ s.e.m.) calculated from the CE vs. CW data illustrated in Panel A.