

Research Article

# The Effects of Proximal and Distal Social Stimulation on Ethanol Intake in Male and Female CD-1 Mice

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**Abstract** *Background.* Proximal and distal social stimulation increase ethanol drinking in humans. *Purpose.* Our study evaluated the effects of proximal and distal social stimulation on home-cage ethanol drinking in mice. *Study design.* Proximal cagemate drinking (PCD) procedures use a clear plastic barrier to separate the drinker mouse from the proximal cagemate mouse, to evaluate home-cage drinking of 10% ethanol and water. Eight groups of CD-1 mice were arranged in a  $2 \times 2 \times 2$  factorial design with two levels of sex of drinker (male vs. female), two levels of sex of cagemate (male vs. female), and two levels of distal group-housed mice in the colony room (present vs. absent). *Results.* Distal group-housed mice, located outside of the home cage, stimulated ethanol drinking in female drinkers and did so regardless of the sex of their proximal cagemate. This effect was observed in the male drinker but only when housed with a proximal male cagemate. *Conclusion.* This study provides the first report of distal social stimulation of ethanol drinking in mice. The distal social stimulation effect, like the effects of proximal social stimulation, was more pronounced in female drinkers.

**Keywords** ethanol; mice; social; housing; cagemate; sex differences

## 1. Introduction

There is considerable evidence that socializing stimulates ethanol drinking in humans and in animals. Humans drink more ethanol in the presence of social interactions than in the absence of such interactions [1,2,3,4]. In humans, there are social factors other than proximal stimulation due to direct visual contact that influence ethanol consumption. For example, distal social stimulation based on perceived normative levels of ethanol consumption has been reported to influence ethanol consumption and in the same manner as the effects of proximal social stimulation [5]. This indicates that social stimulation effects may transcend the physical presence of others provided by direct visual contact.

The stimulating effects of direct proximal social interactions on ethanol intake have also been reported in a number of rodent species, including rats [6,7,8,9,10,11,12,13], mice [14,15,16], and prairie voles [17,18]. It

should be noted that several early investigations employing animal subjects evaluated the effects of social stimulation by comparing ethanol consumption in isolation-housed versus group-housed rodents, and found little evidence of social stimulation of ethanol drinking [7,8,16,19,20]. In their group-housing arrangements, more than one subject was placed in a cage, allowing for direct physical contact among members of the group. Mice and rats that were group-housed with direct physical contact were often reported to drink less ethanol than mice and rats that were housed in isolation [6,8,16]; however, this effect may be due to the distracting effects of physical interference with the opportunity to drink in the home cage. For example, males that are group-housed are likely to show aggression which may distract from drinking ethanol. This seems particularly likely in view of the reports of elevated aggression induced by ethanol drinking in rats [21], and particularly in male rats [22]. It should also be noted that studies employing group-housing arrangements did not employ both males and females in the same group. It would be problematic to place a male and female in direct physical contact with one another, because this may produce mating responses [23,24] which may further interfere with alcohol drinking behavior; therefore, group-housing studies did not employ both males and females at the same time, limiting the range of social stimulation conditions investigated.

Investigators employing proximal cagemate drinking (PCD) procedures have reported that social interaction between males and females induced elevated ethanol drinking in mice [14,15], in rats [6], and in prairie voles [17,25,26]. The earliest study employing PCD procedures [6] evaluated the effects of social housing on ethanol drinking in male rats using a “contact cagemate” procedure where a wire mesh barrier was in place to physically separate the

male drinker from the male cagemate. This study reported that drinking levels of rats in the contact cagemate condition were higher than rats in the group-housed condition, but lower than rats in the isolation control condition [6]. However, this study used only male rats as the drinker and as the cagemate, and therefore did not evaluate the ethanol drinking of females. Nor did it evaluate the effects of social stimulation by males on the ethanol drinking in females, or the effects of social stimulation by females on the ethanol drinking of males.

The current study employed the PCD procedure to study intersex social stimulation effects on ethanol drinking in mice. In our PCD procedure, the drinker mouse is placed in a clear plastic shoebox cage with a clear plastic divider situated lengthwise in the middle. The divider separates the drinker mouse from the cagemate mouse and divides the cage lengthwise into two equal-sized areas. In the PCD procedure, the mice can see and smell each other through holes in the plastic divider, but have restricted physical contact. The PCD procedure allows researchers to study the effects of social stimulation and intersex effects on ethanol drinking without the distracting effects of direct physical contact. Studies have shown that when the PCD procedure is employed and the distracting effects of direct physical interaction are removed, the stimulating effects of social interaction on ethanol drinking are evident [14, 15]. For example, using PCD procedures, mice with one or two proximal cagemates drink more than mice housed in isolation. Note, however, that the double cagemates are group-housed, and they drink less ethanol than their single cagemate counterparts, even though they stimulate more ethanol drinking in the drinker mouse on the other side of the barrier [15].

PCD studies of ethanol drinking in mice have also shown that females are more sensitive to the stimulating effects of direct proximal social interaction on ethanol drinking [14, 15]. In a study placing male or female CD-1 drinker mice in a cage with either zero cagemates (isolation), one cagemate or two cagemates, it was found that females drank more ethanol in the presence of one cagemate, relative to the isolation control, and they drank still more when paired with two cagemates. Male drinker mice, on the other hand, were not as sensitive to the effects of direct proximal social stimulation and exhibited similar levels of ethanol drinking across all three groups [15].

While there are now several investigations reporting effects of proximal social stimulation on ethanol drinking in rodents, there are no studies in rodents evaluating the effects of distal social stimulation on ethanol drinking. The present study asks if these proximal social stimulation effects on ethanol intake in female drinkers and in male drinkers in the PCD procedure are also observed at a distance, with distal social stimulation provided by mice with access to ethanol

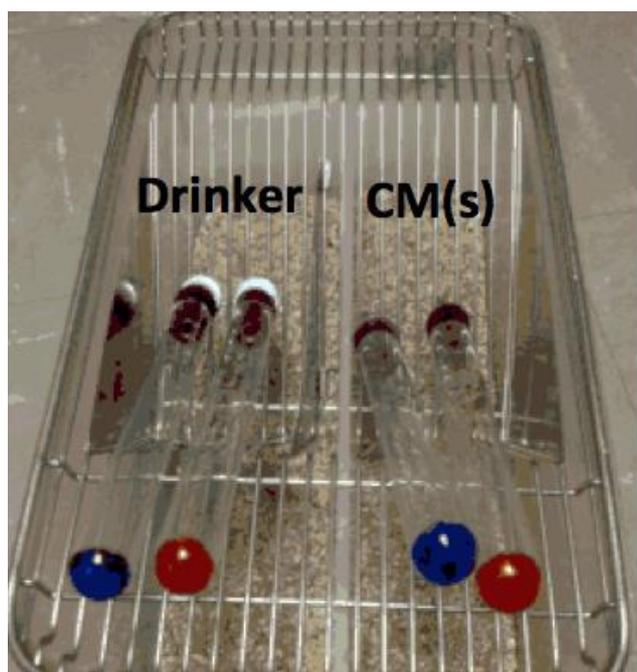
who are group-housed outside of the home cage but inside of the colony room. The presence of these group-housed mice provides social stimulation for the drinker mice, but without direct visual contact. The present study evaluates the effects of distal group-housed mice on ethanol consumption in pairs of PCD-housed mice. This study evaluates the effects of immediate nearby social situations by pairing the drinker mouse with a proximal cagemate mouse housed in the PCD procedure, and, at the same time, the effects of distal social stimulation provided by group-housed mice located in the same colony room but outside of the home cage of the drinker mouse. The study employs a  $2 \times 2 \times 2$  factorial design with two levels of sex of drinker (male vs. female), two levels of sex of cagemate (male vs. female) and two levels of distal group-housed mice condition (present vs. absent).

The hypothesis is that the presence of distal group-housed mice will have influence ethanol drinking of the drinker mice in a manner similar to the effects of proximal social stimulation. That is, distal social stimulation will increase ethanol consumption in the drinker mice, and this effect will be more prominent in female mice since they are more sensitive to social factors and milieu. Additionally, water intake results of the drinkers will be complementary to ethanol intake results, indicating that the stimulating effects of social factors are specific to the drinking of ethanol. Finally, due to the complementary relationship between the intakes of ethanol and water, there will be substantial congruence between ethanol intake results and percent ethanol preference results.

## 2. Materials and methods

### 2.1. Subjects and conditions

The study employed 632 CD-1 mice as drinkers and as cagemates, and in addition, 192 CD-1 mice as distal group-housed mice. All mice were obtained from Charles River (Kingston, NY, USA). CD-1 mice were used because as outbred mice, they are not constructed from specific inbred mouse strains with known genetics variations. Thus, genetic phenomena in human populations such as genetic drift and founder effect are more likely to be present in the outbred mice. In addition, outbred CD-1 mice were employed as experimental subjects because they are known to provide variable levels of ethanol drinking, while also providing stable and modest mean levels of intake of moderate concentrations of ethanol. At the beginning of the study, the mice were approximately 49 days old, and mean body weights were 33.3 g and 26.5 g, for males and females, respectively. Mice were randomly assigned to conditions, as either drinkers or cagemates. Drinkers were 316 CD-1 mice (156 males and 160 females) and cagemates were 316 CD-1 mice (156 males and 160 females). All mice were housed in plastic shoebox cages in a colony room



**Figure 1:** Home-cage arrangement of the PCD procedures. The home cage is divided lengthwise along the middle with a clear plastic barrier separating the drinker from the cagemate. Both the drinker and the cagemate are provided with two sippers. The blue sippers contain water and the red sippers contain 10% ethanol (vol/vol).

with a 12-hour light, 12-hour dark cycle, where they were provided access to food, water, and ethanol ad libitum. All male drinkers paired with a male cagemate were housed in a male-only colony room and all female drinkers paired with a female cagemate were housed in a female-only colony room. All drinkers paired with a cagemate of the other sex were housed in a mixed-sex colony room. There was no direct ventilation between the three colony rooms to prevent odor mixing. All mice were allowed to habituate to their colony rooms for one week prior to the beginning of the experiment. All procedures were performed in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institute of Health and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, 1996) and approved by the IACUC at Rutgers University.

## 2.2. Apparatus

Each drinker mouse was housed with a cagemate mouse in a standard clear plastic shoebox cage equipped with a clear plastic barrier that divided the shoebox cage lengthwise into two equal compartments. The plastic barrier was the height of the shoebox cage and was drilled with 20 quarter-inch

**Table 1:** The table summarizes the  $2 \times 2 \times 2$  factorial design based on all combinations of sex of drinker (male vs. female), sex of cagemate (male vs. female), and distal group-housing conditions (presence of distal group-housed mice with access to ethanol vs. absence of distal group-housed mice). The numbers represent pairs of mice.

		Distal group-housed mice	
		Presence	Absence
Female drinker	Female cagemate	$n = 16$ pairs	$n = 64$ pairs
	Male cagemate	$n = 16$ pairs	$n = 64$ pairs
Male drinker	Female cagemate	$n = 16$ pairs	$n = 60$ pairs
	Male cagemate	$n = 16$ pairs	$n = 64$ pairs

diameter circular holes to allow ventilation between the two compartments. The drinker mouse was placed on the left side of the plastic barrier, while the cagemate mouse was placed on the right side of the plastic barrier. While the drinker and cagemate experienced similar procedures, the designation of the mouse to the left of the barrier as the drinker was arbitrary, but consistent across groups. The mice were able to see and smell each other, but physical interactions were constrained by the presence of the barrier. Both mice were provided with free access to food and to two stainless steel sippers. For each drinker and cagemate mouse one sipper was inserted in the rubber stopper of a glass tube containing ethanol while the other sipper was inserted in the rubber stopper of a glass tube containing water (see Figure 1). In this study, drinker-cagemate pairs were used instead of isolation-housed mice in order to further explore the effects of PCD procedures under evaluation in our laboratory.

## 2.3. Drugs

Bulk ethanol (95%) was obtained from Rutgers University Chemical Stores. Ethanol was diluted in tap water to produce the concentrations (volume to volume, vol/vol) employed in the study.

## 2.4. Ethanol drinking procedures

Subjects were randomly assigned to eight groups. The eight groups were arranged in a  $2 \times 2 \times 2$  factorial design (see Table 1) based on all combinations of sex of drinker (male vs. female), sex of cagemate (male vs. female), and distal group housing conditions (presence of distal group-housed mice vs. absence of distal group-housed mice). Parts of the ethanol intake data from the four groups assigned to the absence of distal group-housed mice condition were presented in a previously published report of a study that included 12 groups of 48 mice per group [14]. These data were combined with and compared to ethanol intake data obtained from eight additional groups ( $n = 16$  mice per group), in order to evaluate the effects of distal social stimulation on ethanol drinking in mice.

The cages for each group in the presence of distal group-housed mice condition were placed in a colony room that included 16 cages of three mice each, one distal drinker and two distal cagemates (i.e., distal group-housed mice). The three mice in each cage had access to ethanol and to water, as described in detail below. Each cage of experimental drinker-cagemate paired mice was placed so as to be at least 2 feet away but no more than 4 feet away from the nearest cage of distal group-housed mice. The distal drinker was separated from the two distal cagemates by a plastic barrier but there was no plastic barrier separating the two distal cagemates. The mice in the male colony room were placed in the presence of 16 cages of distal group-housed male mice. Mice in the female colony room were placed in the presence of 16 cages of distal group-housed female mice. The cages with a male drinker paired with a female cagemate were placed in the presence of 16 cages of distal group-housed mice consisting of a distal male drinker paired with two distal female cagemates. The cages with a female drinker paired with a male cagemate were placed in the presence of 16 cages of the distal group-housed mice consisting of a distal female drinker paired with two distal male cagemates.

The PCD procedures were employed during each daily 23-hour drinking session, with 1-hour time set aside for mouse weighing, liquid consumption recording, and refilling of drinking tubes for the drinker mice. Time constraints did not allow for all of these procedures to be completed for the cagemates; therefore, drinking data were obtained only for the drinkers. During each of the 12 daily drinking sessions, each drinker mouse was weighed daily at approximately 1000 h. Each of the four groups of drinker and cagemate mice in the presence of distal group-housed mice condition was placed in a room containing group-housed mice provided with access to ethanol and water. Each of the four groups in the absence of group-housed mice condition was placed in a colony room containing the experimental subjects, and no distal group-housed mice. During experimental days 1–5, the drinker mice and the cagemate mice as well as the distal group-housed mice were provided with access to ethanol, the vol/vol. concentration of ethanol in the sipper was increased daily in 2% increments from 2% to 10%, and then maintained at 10% for the remaining seven days of the study (experimental days 6–12). This schedule of ascending ethanol concentrations, with a maximum ethanol concentration of 10%, was similar to the schedule of ethanol concentrations employed in earlier investigations of intersex effects in rats [11] and in prairie voles [26]. For the drinker mice, fluid bottles were weighed, emptied, refilled, and reweighed during the 1-hour set-aside time, to allow determination of the amount of fluid removed from each bottle during each 23-hour drinking session. For the

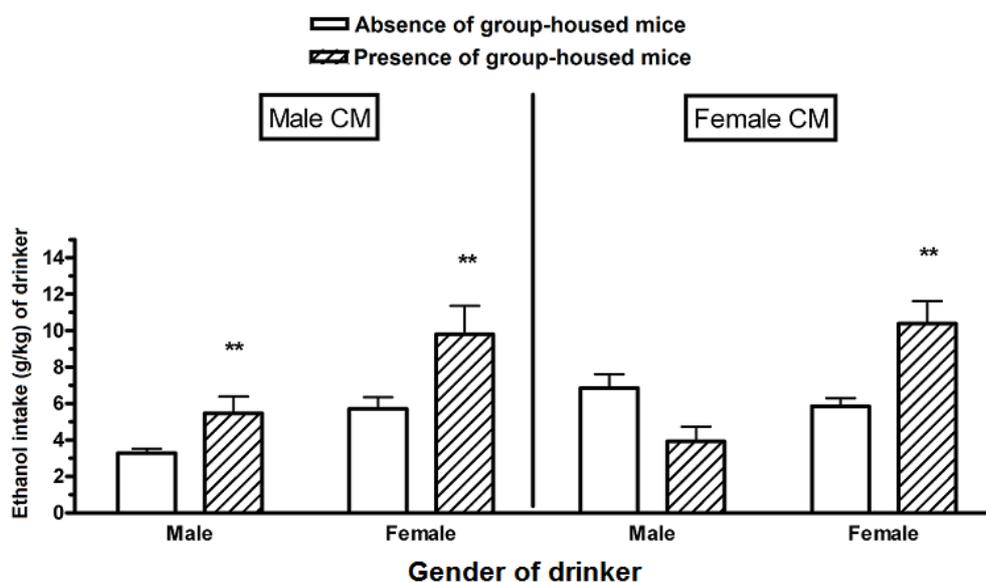
cagemate mice, fluid bottles were emptied and refilled, but were not weighed or reweighed during the 1-hour set-aside time due to time constraints; therefore, daily levels of drinking of ethanol and water of the cagemate mice were not recorded. The positions of the ethanol bottle and the water bottle in each cage were randomized across days for drinker mice and for cagemate mice.

### 3. Results

For each drinker mouse, for each daily session, ethanol fluid consumed (g), water fluid consumed (g), and bodyweight (g) were measured. For each drinker mouse, grams of ethanol consumed per kg of bodyweight (g/kg ethanol intake), grams of water consumed per kg of bodyweight (g/kg water intake) and percent ethanol preference (grams of ethanol fluid consumed divided by the sum of grams of ethanol fluid consumed plus grams of water consumed) were derived. For each subject, the mean for each measure (g/kg ethanol intake, g/kg water intake, % ethanol preference) of the last five daily sessions of training with the 10% ethanol solution (sessions 8–12) was derived. ANOVAs revealed that there were no statistically significant effects of sessions (all  $P$ 's  $> .10$ ); therefore, each of these five-day means provided the data subjected to statistical analysis. For the eight groups, the effects of sex of drinker (male vs. female), sex of cagemate (male vs. female), distal group-housed mice condition (presence of distal group-housed mice vs. absence of distal group-housed mice), and their interaction effects were assessed by three-way  $2 \times 2 \times 2$  univariate analysis of variance (ANOVA) using General Linear Model (Systat Statistical Software, Richmond, CA, USA), with a two-tailed alpha level of 0.05. Note that a significant two-way interaction between sex of drinker and sex of cagemate is revealed by a significant main effect of sex pairing arrangement (same sex pairing vs. other sex pairing). A significant three-way interaction was followed by  $2 \times 2$  ANOVA using sex pairing arrangement (same sex pairing vs. other sex pairing) and distal group-housed mice condition as factors. For each sex of drinker, a  $2 \times 2$  ANOVA evaluated the effects of sex of cagemate and distal group-housed mice. Planned comparisons evaluated the effects of sex of cagemate under each location of distal group-housed mice condition.

#### 3.1. Overall analyses of ethanol intake

Group mean differences in daily grams per kilogram ethanol intake during the last five days of the experiment for the eight groups of drinkers were evaluated using a three-way  $2 \times 2 \times 2$  ANOVA (see Figure 2). The analysis revealed that the ethanol intake of the female drinkers was significantly elevated relative to the ethanol intake of male drinkers ( $F[1,308] = 24.485$ ,  $P < .01$ ). There was a statistically significant elevating effect of the presence of distal group-housed mice, ( $F[1,308] = 10.110$ ,



**Figure 2:** Group mean ethanol intake (g/kg) per drinker during the last five drinking sessions when the sipper contained 10% ethanol (vol/vol) for the CD-1 mice. The vertical bars represent the standard error of the mean (SEM). The double asterisk (\*\*) above the column indicates that the group difference relative to the adjacent column was significant ( $P < .01$ ).

$P < .01$ ), indicating that the subjects in the presence of distal group-housed mice condition drank more ethanol than the subjects in the absence of distal group-housed mice condition. There was no significant main effect of sex of cagemate ( $F[1,308] = 1.272, P > .05$ ), and there was no significant interaction between sex of drinker and sex of cagemate ( $F < 1$ ), indicating that the ethanol intake of same sex pairings did not differ from the ethanol intake of other sex pairings. The elevating effect of the presence of distal group-housed mice depended on the sex of the drinker ( $F[1,308] = 14.275, P < .01$ ), but did not interact significantly with the sex of the cagemate ( $F[1,308] = 3.574, P > .05$ ), indicating that the presence of distal group-housed mice stimulated ethanol drinking, particularly in female drinkers, and did so regardless of the sex of the cagemate. The three-way interaction between sex of the drinker, sex of the cagemate, and distal group-housed mice condition was also significant ( $F[1,308] = 5.008, P < .05$ ).

One-way  $1 \times 2$  ANOVA revealed that the presence of distal group-housed mice, using sex of cagemates (both sexes combined), did not significantly elevate ethanol drinking in the male drinker ( $F < 1$ ). However, this effect was significant in the female drinker ( $F[1,154] = 22.070, P < .01$ ), indicating that females were more sensitive to the external social stimulation provided by the presence of distal group-housed mice.

### 3.1.1. Ethanol intake of the male drinker

The  $2 \times 2$  ANOVA for the four groups of male drinkers revealed no significant main effect of the sex of the cagemate

( $F[1,152] = 1.526, P > .05$ ), no significant main effect of distal group-housed mice condition ( $F < 1$ ), but a significant interaction between the sex of the cagemate and distal group-housed mice condition ( $F[1,152] = 9.611, P < .01$ ). One-way  $1 \times 2$  ANOVA revealed that in the case of a male drinker housed with a male cagemate, the effect of the presence of group-housed mice was to significantly elevate ethanol intake ( $F[1,78] = 10.178, P < .01$ ); however, the opposite trend was observed with the female cagemate, where the effect of the presence of group-housed mice was to nonsignificantly reduce the ethanol intake of the male drinker ( $F[1,74] = 3.708, .10 > P > .05$ ). For the male drinker housed in the absence of distal group-housed mice, there was a significant main effect of the sex of the cagemate ( $F[1,122] = 21.317, P < .01$ ). This indicates that in the absence of distal group-housed mice, the female cagemate was significantly more effective in stimulating ethanol drinking in the male drinker than was the male cagemate; however, this effect was not evident when in the presence of distal group-housed mice ( $F < 1$ ).

### 3.1.2. Ethanol intake of the female drinker

The  $2 \times 2$  ANOVA for the four groups of female drinkers revealed no significant main effect of the sex of the cagemate ( $F < 1$ ), a significant main effect of distal group-housed mice condition ( $F[1,156] = 21.185, P < .01$ ), and no significant interaction between the sex of the cagemate and presence of distal group-housed mice condition ( $F < 1$ ). Females drinkers housed with a male cagemate drank more ethanol in the presence of distal group-housed mice than they did in the absence of distal group-housed mice

( $F[1, 78] = 7.443, P < .01$ ). Female drinkers housed with a female cagemate also drank more in the presence of distal group-housed mice than they did in the absence of distal group-housed mice ( $F[1, 78] = 17.760, P < .01$ ).

### 3.2. Water intake and percent ethanol preference of the drinker mice

The two-way  $2 \times 2$  ANOVA revealed that the effects of the distal group-housed mice condition on group mean g/kg water intakes were complementary to their effects on ethanol intake. That is, the presence of distal group-housed mice significantly reduced, rather than significantly increased, group mean water intakes (data not shown). The disparities between the water intake results and ethanol intake results indicate that the observed effects of distal group-housed mice on the ethanol intakes of drinkers was specific to ethanol intake and, therefore, unlikely due to nonspecific factors. Finally, it should be noted that the complementary relationship between mean ethanol intakes and mean water intakes in the drinkers contributed to the substantial congruence between the patterns of mean ethanol intakes and mean percent ethanol preferences (data not shown).

## 4. Discussion

Our results revealed that the presence of cages of group-housed mice in the colony room influenced the ethanol drinking of experimental subjects housed nearby in that colony room. The effect of the distal group-housed mice was to stimulate ethanol intake in female drinkers, and this was the case regardless of the sex of the cagemate paired with the female drinker. In male drinkers, this effect of the distal group-housed mice was observed only when paired with a male cagemate, but not when the male drinker was paired with a female cagemate. We are not aware of any previous studies reporting effects in animals of distal group-housing on ethanol drinking; therefore, the present study provides the first report of this type of social stimulation effect. The results provide useful information about social stimulation on ethanol drinking because of the similar effects on ethanol drinking in proximal and distal stimulation situations, lending support to the conclusion that social stimulation is not carried by visual stimulation only.

### 4.1. Ethanol intake of the female drinkers

The present study employed PCD procedures to evaluate the effects of distal group-housed mice on ethanol intake, water intake, and percent ethanol preference of the drinker mouse. The results revealed that the distal group-housed mice stimulated ethanol drinking in the female drinker mouse. In the presence of distal group-housed mice, female drinkers consumed significantly more ethanol and significantly less water than female drinkers in the absence of distal group-housed mice condition. That is, female drinkers housed in

the colony room in the presence of either distally group-housed males or distally group-housed females consumed significantly more ethanol than female drinkers housed in a colony room without distal group-housed mice. The finding that the ethanol drinking of female mice is stimulated by social factors is consistent with a PCD study that found that a female drinker mouse housed with two cagemates consumed more ethanol than a female drinker mouse housed with one cagemate or a female drinker mouse housed in isolation [15].

### 4.2. Ethanol intake of the male drinkers

In contrast to their robust effects on the female drinker, the distal group-housed mice did not consistently influence the ethanol intake of the male drinker. Male mice in the presence of distal group-housed mice condition consumed more ethanol only when the proximal cagemate mouse was also a male. However, when the proximal cagemate mouse was a female, there was no statistically significant effect of the distal group-housed mice condition.

It should be noted, however, that the pattern of results of a male drinker housed with a male cagemate is opposite to the pattern shown when a male drinker is housed with a female cagemate, even though in the latter case the mean difference failed to achieve statistical significance. That is, when a male drinker was housed with a male cagemate, the male drinker consumed significantly more ethanol in the presence of distal group-housed mice than it did in the absence of distal group-housed mice. However, when a male drinker was housed with a female cagemate, the male drinker consumed nonsignificantly more ethanol in the absence of distal group-housed mice than it did in the presence of distal group-housed mice. These effects, however, contributed to the statistically significant interaction between sex of cagemate and distal group housing condition in male drinkers.

Additionally, in the absence of group-housed mice, the male drinker paired with the female cagemate consumed significantly more ethanol than the male drinker paired with a male cagemate. This pattern of results is similar to the results observed in a previous PCD study employing a single cagemate, where a single female cagemate induced elevated ethanol intake in the male drinker relative to pairing with a single male cagemate [15].

The pattern of results observed in male drinkers suggests that the effect of distal group-housed mice depends on the sex of the mice providing the social stimulation. Distal group-housed males significantly elevated ethanol intake in male drinkers, which is reminiscent of "the Greek effect" seen in studies of elevated ethanol drinking in fraternity houses [27, 28, 29, 30]. On the other hand, distal group-housed females actually reduced, though nonsignificantly, ethanol intake in male drinkers, suggesting

that the distal group-housed females may have served to distract the males from drinking ethanol. This is consistent with the findings of a previous PCD study where female cagemates nonsignificantly reduced ethanol intake of male drinkers, relative to isolation-housed controls [15] and two female cagemates significantly reduced ethanol intake of male drinkers relative to the single female cagemate condition [15].

#### 4.3. Sex differences in ethanol intake

The results of the present study indicate that female mice drink more ethanol than male mice and they do so in a number of different social situations. This pattern of sex differences in ethanol drinking is consistent with studies reporting that isolation-housed female mice and rats produced elevated ethanol intake relative to isolation-housed male mice and rats [31,32,33,34,35,36]. The present results add to a growing body of literature indicating that these sex differences are not specific to drinking ethanol in isolation [14, 15]; see also [31].

#### 4.4. Effects of distal versus proximal social stimulation

How do the effects of distal social stimulation observed in the present study compare to the effects of proximal social stimulation reported in an earlier PCD study [15]? There are several similarities. For example, distal group-housed females (distal social stimulation) and female cagemates (proximal social stimulation) significantly increased ethanol intakes of the female drinkers. Moreover, distal group-housed females were similar to female cagemates in that neither had an effect on ethanol intakes of the male drinkers, indicating that the effect of distal females was similar to the effect of proximal females. On the other hand, the effect of distal males did not resemble the effect of proximal males. Distal group-housed males significantly increased ethanol intakes of female drinkers and male drinkers, but proximal male cagemates had no effect on ethanol intakes of either female drinkers or male drinkers, indicating that with respect to ethanol intake, for male drinkers and for female drinkers, two distal group-housed males stimulated ethanol intake but two proximal males did not.

#### 4.5. Implications of the present study

The results of the present study detail the effects of distal social stimulation on ethanol drinking in male and female mice. The effects of distal social stimulation significantly influenced the effectiveness of the proximal cagemate in stimulating the drinker to consume ethanol. For example, the male cagemate was significantly more effective in stimulating ethanol intake of the drinker when in the presence of distal group-housed mice, and this effect was observed with the male drinker and with the female drinker. The female cagemate was significantly more effective in

stimulating ethanol intake of the female drinker when in the presence of distal group-housed mice, but this effect was not observed with the male drinker.

The effects of the presence of the group-housed mice condition on the ethanol drinking of the male drinker are particularly important because of its implications for interpreting the results of previous studies reporting the effects of isolation versus group housing. These studies have largely employed only males, and, in the present study the male drinker housed with the male cagemate was shown to be affected by distal social stimulation. Previous studies employing males to test the effects of isolation versus group housing have not detailed the housing conditions in the colony room, and this may have contributed to the discrepant findings in the literature. For example, several studies report an effect of group-housed male mice versus male mice housed in isolation, such that male mice in isolation consumed more ethanol than their group-housed counterparts [7,8,32]. However, several other studies show an opposite effect or no effect [37,38]. The results of the present study reveal the need for attention to details regarding the housing arrangements employed in the colony room, in order to clarify the possible effect of distal social stimulation provided by distal group-housed mice provided with access to ethanol.

Many studies designed to examine the effects of isolation versus social stimulation on the ethanol drinking rodents have utilized group housing, in which the animals are housed in direct physical contact with one another [7, 8,16,32,39]. Allowing group-housed animals to share the colony room with isolation-housed controls may produce an effect similar to that of the distal group-housed mice. Future studies should take into consideration this possible effect, as distal group-housed animals can serve as social stimulation and influence ethanol drinking in other cages located within the same colony room.

It should be noted that the effects of distal group-housing on ethanol intake in male or female drinkers cannot be attributed to nonspecific factors such as arousal-induced drinking [40,41] or adjunctive polydipsia [42,43, 44], as analysis of group mean water intake data revealed results that were complementary to group mean ethanol intake data. That is, the groups of drinkers that provided higher levels of ethanol intake tended to provide lower levels of water intake. The complementary relationship in drinkers between ethanol intake and water intake was observed in male drinkers and in female drinkers, as well as when these drinkers were paired with a male cagemate or when paired with a female cagemate. The complementary relationship between ethanol intake and water intake in drinkers contributes to the substantial congruence between the patterns of ethanol intake and percent ethanol preference in this study.

To further explore the effects of distal group-housed mice on ethanol consumption in mice, it will be informative that future studies test the effects of distal group-housed mice on male and female mice housed in isolation compared to mice housed with a proximal cagemate. In addition, future studies will test the importance of the distal group-housed mice's access to ethanol by conducting experiments in which the distal group-housed mice are given either ethanol and water, or only water. Results from PCD studies conducted in our lab suggest that the availability of ethanol to the social stimulus is a prominent factor in the social stimulating effects of the proximal cagemate [14].

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**Conflict of interest** The authors declare that they have no conflict of interest.

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