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Effects of pair housing on voluntary alcohol intake in male and female Wistar rats

Hayden Scott [a, b, 1], Nikita Tjernström [a, 1], Erika Roman [a, c, *, 2]

[a] Department of Pharmaceutical Biosciences, Research Group Neuropharmacology Addiction and Behavior, Uppsala University, Uppsala, Sweden
[b] Department of Brain and Cognitive Sciences, University of Rochester, Rochester, NY, United States
[c] Department of Anatomy, Physiology and Biochemistry, Division of Anatomy and Physiology, Swedish University of Agricultural Sciences, Uppsala, Sweden

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ABSTRACT

A number of different voluntary alcohol intake paradigms are available for home cage drinking studies. Traditionally, these paradigms involve single housing in order for individual intake to be measured. This study aimed at investigating the effects of pair housing on voluntary alcohol intake. Male and female Wistar rats were housed in pairs or individually for studies of voluntary alcohol intake using the modified intermittent access paradigm with alcohol access during three consecutive days per week followed by four days of water only. Individual intake of 20% alcohol solution and water was measured during 12 sessions, i.e., 4 weeks. Pair-housed animals could interact freely with their cage mate for four consecutive days each week and were then separated by an inserted mesh divider for three consecutive days each week during alcohol intake sessions. Alcohol intake and preference were compared between pair-housed and individually housed rats. The results revealed higher alcohol intake in females than in males. Pair-housed males had a higher alcohol intake and preference during the first 3 weeks, but not during the fourth week, compared to individually housed males No effect of housing condition was observed in female rats. The alcohol intake was higher on the first day of access relative to the two consecutive days in pair-housed males and higher on the first two days relative to the third day in female rats. Social rank or female estrus cycle had no effect on alcohol intake or preference. Taken together, the use of a divider during alcohol intake sessions had no impact on alcohol intake in female rats and may not exert long-term influences in male rats. Future studies are needed in order to elucidate whether the use of a divider can constitute an experimental refinement as an alternative to individual housing in studies of voluntary alcohol intake using the limited access and/or intermittent access paradigms.

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Introduction

Alcohol is among the most widely used drugs of abuse, and excessive consumption of alcohol is a global leading risk factor for morbidity, disability, and mortality (GBD 2016 Alcohol Collaborators, 2018). Animal models on voluntary alcohol (i.e., ethanol) consumption have a long-standing tradition in preclinical research on alcohol use disorders (AUDs), with good face and construct validity (Becker, 2013; Becker & Koob, 2016; Crabbe, 2014; Meisch, 2001; Sanchis-Segura & Spanagel, 2006). Like humans, outbred rats display large individual differences in voluntary alcohol intake, intake patterns, and the development of excessive alcohol intake (Meisch, 2001; Momeni & Roman, 2014; Palm, Roman, & Nylander, 2011; Simms et al., 2008; Spoelder et al., 2017; Steensland et al., 2012; Wise, 1973; Wolffgramm, 1990).

In preclinical research, several different voluntary alcohol intake paradigms are used, including continuous, limited, and intermittent access, which are all based on a free choice between alcohol solution(s) and water in the home cage (Becker, 2013; Carnicella, Ron, & Barak, 2014; Meisch, 2001; Sanchis-Segura & Spanagel, 2006). The intermittent access paradigm for voluntary alcohol intake is based on repeated cycles of alcohol access and access to water only (Becker, 2013; Carnicella et al., 2014; Simms et al., 2008;

* Corresponding author. Department of Pharmaceutical Biosciences, Research Group Neuropharmacology Addiction and Behavior, Uppsala University, P.O. Box 591, 751 24 Uppsala, Sweden. Tel.: +46 18 4714632.
E-mail addresses: Erika.Roman@farmbio.uu.se, Erika.Roman@slu.se (E. Roman).
[1] Equal contribution from both authors.
[2] Department of Anatomy, Physiology and Biochemistry, Division of Anatomy and Physiology, Swedish University of Agricultural Sciences, P.O. Box 7011, 750 07, Uppsala, Sweden. Tel.: +46 18 672843.

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Wise, 1973). The use of a removable cage divider, which allows for normal interaction with the cage mate except during alcohol intake sessions when individual intake measures can be collected, has been introduced (Ryabinin & Walcott, 2018). This approach has primarily been used for studies of voluntary alcohol intake in adolescent animals (Fernandez et al., 2017; Palm & Nylander, 2014; Vetter-O'Hagen, Varlinskaya, & Spear, 2009; Wille-Bille et al., 2017). For instance, when a divider was used in studies of voluntary alcohol intake in adolescent male Wistar rats during intermittent 2-hour alcohol intake sessions, no difference in alcohol intake and preference was found between pair-housed and individually housed adolescent males (Palm & Nylander, 2014). However, to the best of our knowledge, it is not known whether the use of a mesh divider affects voluntary alcohol intake in adult rats with 24-hour access. Therefore, this study sought to investigate whether adult pair-housed male and female Wistar rats differed in their alcohol intake behavior from individually housed rats in a modified intermittent access paradigm (Lundberg, Abelson, Nylander, & Roman, 2017; Momeni & Roman, 2014; Momeni, Segerstrom, & Roman, 2015; Tjernstrom & Roman, 2018) with alcohol access during three consecutive days per week followed by four days of water only.

Material and methods

Animals

Forty outbred Wistar rats (20 males and 20 females; RccHan:WI, Envigo [former Harlan Laboratories B.V.], Horst, the Netherlands) were delivered to the animal facility at 7 weeks of age. Upon arrival, the animals were randomly housed in pairs in transparent type IV cages (59 cm × 38 cm, height 20 cm) with raised cage lids containing wood-chip bedding and two sheets of paper (40 cm × 60 cm; Cellstoff, Papyrus) as enrichment. The cages were placed in temperature- (21 ± 1 °C) and humidity- (50 ± 10%) controlled cabinets in an animal room on a reversed light/dark cycle (lights off at 6:00 AM) with a masking background noise. Male and female rats were housed in the same room but in different cabinets. Throughout the experiment, the animals were maintained on standard rat chow (R36, Lantmännen, Kimstad, Sweden) and water *ad libitum*. After arrival, the rats were given an undisturbed 2-week acclimatization period (Arts, 2016) to adjust to the facility and the reversed light/dark cycle. All animal experiments were approved by the Uppsala Animal Ethical Committee (C14/15) and followed the guidelines of the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS 1998:56) and the European Union Directive on the Protection of Animals Used for Scientific Purposes (Directive 2010/63/EU).

Voluntary alcohol intake

Following the acclimatization period, all animals were marked by ear punching. During the week prior to alcohol access, all rats were individually handled and weighed in order to habituate to the experimenter. At 10 weeks of age, the animals were separated into two different housing conditions (each with 10 males and 10 females) – continued pair-housing with the familiar cage mate in transparent type IV cages (59 cm × 38 cm, height 20 cm) with raised cage lids, or individual housing in transparent type III high cages (42.5 cm × 26.5 cm, height 18 cm) with flat cage lids as used in previous experiments (Lundberg et al., 2017; Momeni & Roman, 2014; Momeni et al., 2015; Tjernstrom & Roman, 2018). The cages contained wood-chip bedding, and both groups had two sheets of paper (40 cm × 60 cm; Cellstoff, Papyrus) per week as enrichment. The rats were given access to alcohol by a modified intermittent two-bottle free-choice (20% v/v alcohol solution and water) paradigm with alcohol access during three consecutive days (Tuesdays–Thursdays) per week followed by four days of water only (Lundberg et al., 2017; Momeni & Roman, 2014; Momeni et al., 2015; Tjernstrom & Roman, 2018). For the pair-housed rats, a divider was used to allow for individual intake measurements. The divider was inserted just prior to alcohol access and removed at the end of the last session, i.e., after the third consecutive 24-hour session. Thus, the rats had four consecutive days of social interaction every week. The divider (Fig. 1) is made of transparent plastic with a wire mesh section to allow some tactile contact on alcohol-access days (Palm & Nylander, 2014). Notably, when the divider was inserted in the cage, each rat had individual food, and alcohol and water bottles, and the rats were not able to sample alcohol from the other rat in a pair. Moreover, with the divider inserted, the bottom area of the cage for each individual rat in a pair was equivalent to that of the individually housed rats. Alcohol solution (diluted in tap water from 96% ethanol, Solveco Etanol A 96%; Solveco AB, Rosersberg, Sweden) and tap water were provided in 150-mL bottles with ball valve nipples (Scanbur AB, Sollentuna, Sweden) with minimal spillage. Fresh alcohol solution and water at room temperature were provided for every 24-hour session, and bottle positions were rotated to avoid potential side bias. Between alcohol sessions, the animals had access to one bottle of tap water. Individual alcohol and water intake were measured every 24 hours during the three access days by weighing the bottles for a total of 12 sessions, i.e., 4 weeks. Alcohol intake (g/kg), alcohol preference (% of total fluid intake), water intake (g/kg), and total fluid intake (g/kg) were calculated for each session. To minimize disturbance during alcohol intake sessions, the animals were weighed and cages with paper sheets were changed once a week on a day with access to water.

Estrous cycle

Vaginal smears were collected at 9:00 AM on Mondays–Fridays during the weeks of access to alcohol to analyze the influence of estrous cycle on female drinking behavior. No corresponding handling of male rats occurred on the days when the divider was in place in the cages. On alcohol-access days, the vaginal smears were collected prior to alcohol access. A plastic pipette was used to flush a small amount of water into the vagina, which was then subsequently retrieved and transferred to a slide and left to dry in room temperature. The smears were then stained using a May-Grünwald-Giemsa protocol (Roman, Gustafsson, Hyytia, & Nylander, 2005), in which the May-Grünwald solution stains the cytoplasm

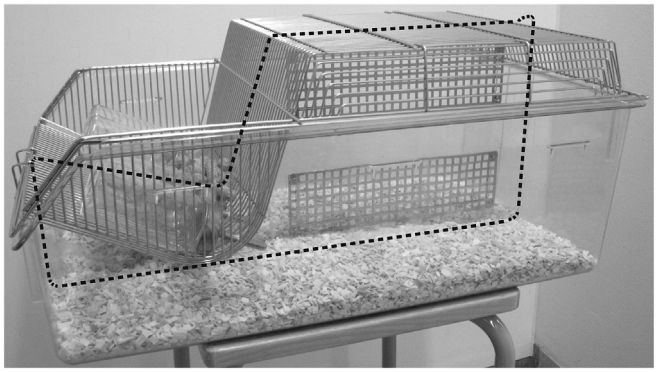

Fig. 1. A type IV cage (59 cm × 38 cm × 20 cm) with the divider, highlighted by the dashed line, fitted under the raised cage lid (Palm, 2014), enabling individual intake measurements in pair-housed rats.

and granule, and the Giemsa stains the nuclei and cytoplasm. In brief, dry smears were fixed in methanol (5 minutes at room temperature), transferred to May-Grünwald solution (5 minutes at room temperature), and finally to Giemsa's stain solution for 15 minutes (1:50 in distilled water at room temperature; all solutions from VWR, Stockholm, Sweden). The smears were classified by dominating cell type under a microscope (× 100 magnification) as proestrus (nucleated epithelial cells), estrus (cornified epithelial cells), or diestrus (leukocytes) by an experimenter blind to housing conditions.

Social rank

Social rank was investigated in order to assess the influence of social rank on drinking behavior in the pair-housed rats. Two approaches were used, a modified version of the tube test originally described for mice (Lindzey, Winston, & Manosevitz, 1961) and in recent years adopted for studies in rats (Jupp et al., 2016; Saxena et al., 2018), and the cruder measure of body weight (Agren, Lund, Thiblin, & Lundeberg, 2009; Pohorecky, 2008).

In the tube test, an animal's choice to advance or retreat following an interaction with a competitor within the tube is assessed. A grey plastic PVC tube of sufficient size to allow one but not two rats to move through the tube was used (inner diameter 7.0 cm for male rats and 4.5 cm for female rats, length 100 cm). The rats within a pair were simultaneously released into opposite ends of the tube. The rat within each pair that remained in the tube or that managed to travel through the tube was deemed dominant while the rat that retreated was designated subordinate. The test was repeated three times for each pair and entry ends were switched for each time. The test was conducted on the fourth day of access to water only in order to exclude any influence of alcohol on the performance. Males and females were tested separately.

The cruder measure of body weight assumes that individuals of higher social rank tend to weigh more (Agren et al., 2009; Pohorecky, 2008). Hence, the individual within each pair with the higher body weight over time was considered dominant over the individual with the lower body weight.

Statistical analyses

Statistical analyses were carried out in R 3.2.3 (R Core Team, 2015) using the nparLD package (Noguchi, Gel, Brunner, & Konietschke, 2012) for non-parametric analysis of longitudinal data in factorial experiments, and in Statistica 13.2 (Dell Inc., Tulsa, Oklahoma, United States). Parameters were examined for normality using the Shapiro–Wilk's W test. Body weight data were found to have a normal distribution and parametric statistics were used. Main effects and interactions were examined using repeated-measures ANOVA with housing condition and sex as between-subject factors, and time as a within-subject factor. Intake data did not show a normal distribution and consequently non-parametric statistics were used. Main effects and interactions of longitudinal drinking data were examined using the R package nparLD (Noguchi et al., 2012) with housing condition and sex as between-subject factors, and time as a within-subject factor. For this analysis, missing values in the drinking data set were imputed as the average of the flanking sessions for that individual. Group-dependent *post hoc* tests were performed with the Mann–Whitney U test with continuity correction, and time-dependent *post hoc* tests were performed with the Wilcoxon's matched pairs test. In the analysis of alcohol intake and preference for access days 1, 2, and 3, the first week of access was excluded since alcohol was novel, and the average intake and preference across weeks 2–4 were used. The influence of social hierarchy on intake data was analyzed with the Mann–Whitney U test with continuity correction, and the influence of estrus cycle phase was analyzed with the Kruskal–Wallis ANOVA by ranks followed by the Mann–Whitney U test with continuity correction when appropriate. Within-subject effects of the estrus cycle on intake data were investigated using the Friedman test followed by the Wilcoxon's matched pairs test when appropriate. Data were considered statistically significant at $p < 0.05$.

Results

Body weight

The body weight data during the 4 weeks of voluntary alcohol intake are shown in Fig. 2. A main effect of sex [$F(1,36) = 424.5$, $p < 0.001$) and time [$F(3,108) = 523.0$, $p < 0.001$), respectively, and an interaction between sex and time [$F(3,108) = 110.8$, $p < 0.001$) was found, with higher body weight in males than in females (Tukey HSD $p < 0.001$). No effect of housing condition on body weight was revealed [$F(1,36) = 0.1$, $p = 0.78$).

Main effects of sex

There was a significant main effect of sex for voluntary alcohol intake, water intake, and total fluid intake, but not for alcohol preference (Supplementary Table 1). As shown in Fig. 3, the alcohol intake during weeks 1, 2, and 4 was higher in females than in males, independent of housing condition. Moreover, except for the first session, the water intake and weekly water intake were higher in females than in males, independent of housing condition (Supplementary Table 2). Finally, the total fluid intake and weekly total fluid intake were higher in females than in males, independent of housing condition (Supplementary Table 2). For simplicity, intake and preference data are presented separately for males and females below.

Effects of housing condition on voluntary alcohol intake and preference

The alcohol intake and preference during the 12 sessions of alcohol access are shown in Fig. 4. There was a main effect of sex, housing condition, and session, as well as an interaction between housing condition and session on alcohol intake (Supplementary

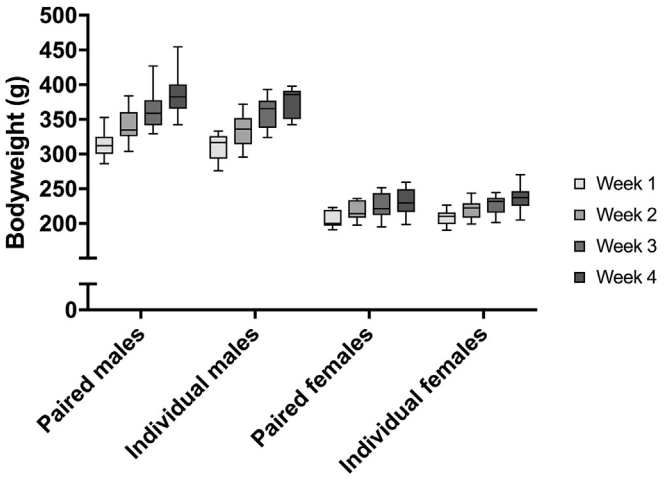

Fig. 2. The mean body weight (g) of male and female pair-housed and individually housed rats during the 4 weeks of voluntary alcohol intake.

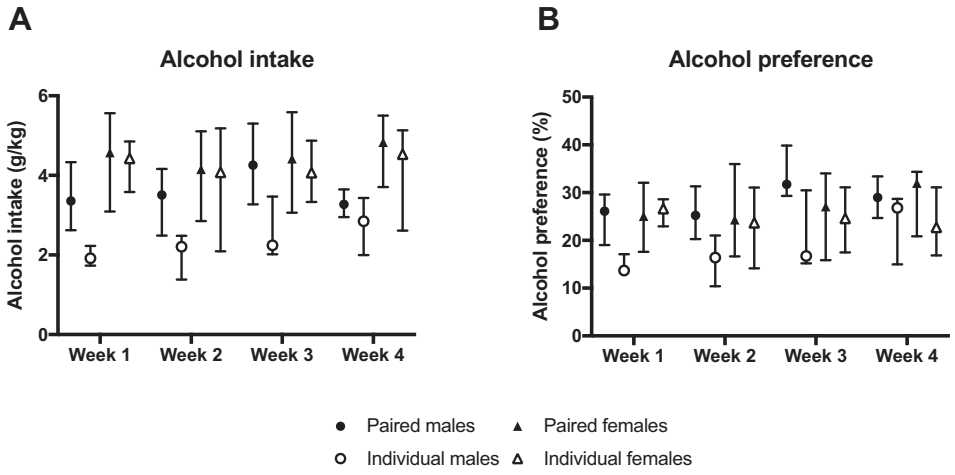

Fig. 3. Weekly average alcohol intake (g/kg; **A**) and preference (%; **B**) in male and female rats during the 12 sessions, i.e., 4 weeks, of voluntary alcohol intake using the modified intermittent access paradigm with a free choice between 20% alcohol and water for three consecutive days and four days of water only. Pair-housed rats were separated using a divider during the alcohol intake sessions in order to retrieve individual intake measurements, and individually housed rats were single-housed throughout the 4 weeks. Data are presented as median and interquartile range (n = 10/group). There was a significant main effect of sex (see Supplementary Tables 1-2 for details) for voluntary alcohol intake, but not preference with higher alcohol intake in females than in males (R package nparLD).

Table 1). With regard to housing condition, pair-housed males had a higher alcohol intake during sessions 1–5, and during session 7 than individually housed male rats (Fig. 4A). Moreover, pair-housed males had a higher alcohol intake than individually housed males during weeks 1–3 (Supplementary Table 3). In contrast, no effect of housing condition on alcohol intake was found in female rats (Fig. 4B, Supplementary Table 3).

With regard to alcohol preference during the 12 sessions of alcohol access, there was a main effect of housing condition and session, as well as an interaction between sex and session (Supplementary Table 1). With regard to housing condition, pair-housed males had a higher alcohol preference during sessions 1–4 and 7 than individually housed male rats (Fig. 4C). Moreover, pair-housed males had a higher alcohol preference than

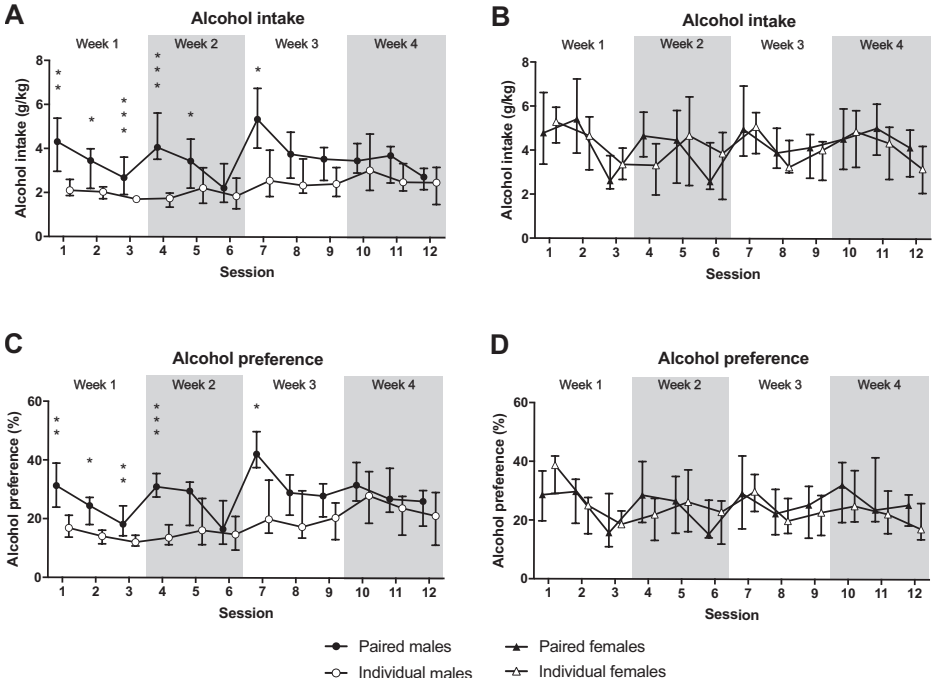

Fig. 4. Alcohol intake (g/kg) and preference (%) in male (**A** and **C**, respectively) and female (**B** and **D**, respectively) rats during the 12 sessions of voluntary alcohol intake using the modified intermittent access paradigm with a free choice between 20% alcohol and water for three consecutive days and four days of water only. Pair-housed rats were separated using a divider during the alcohol intake sessions in order to retrieve individual intake measurements, and individually housed rats were single-housed throughout the 4 weeks. Data are presented as median and interquartile range (n = 10/group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to individually housed animals within the respective sex (Mann–Whitney U test).

individually housed males during weeks 1–2 (Supplementary Table 3). In contrast, no effect of housing condition on alcohol preference was found in female rats (Fig. 4D, Supplementary Table 3).

Differences upon regaining alcohol access (drinking day 1) compared to when accustomed (drinking day 3) were assessed by comparing the average alcohol intake (Fig. 5A) and preference (Fig. 5B) for access days 1, 2, and 3 across weeks 2–4. Pair-housed males had a higher alcohol intake on the first day of access than individually housed male rats (Fig. 5A), while no effect of housing condition was observed in female rats (Fig. 5A). The intake within the respective group showed an evident pattern in pair-housed male rats with the highest intake on day 1 relative to days 2 and 3. In individually housed males, no difference across days was observed (Fig. 5A). In females, the pattern was similar in both groups, with a higher intake on days 1–2 relative to day 3 (Fig. 5A). With regard to alcohol preference, pair-housed males had a higher alcohol preference on the first day of access than individually housed male rats (Fig. 5B), while no effect of housing condition was observed in female rats (Fig. 5B). The preference within the respective group showed a pattern consistent with that of alcohol intake, with the highest preference on day 1 relative to days 2 and 3 in pair-housed male rats, while no difference across days was observed in individually housed males (Fig. 5B). In the females, the preference was higher on day 1 relative to days 2 and 3 in the pair-housed group, and higher on days 1–2 relative to day 3 in the individually housed group (Fig. 5B).

Effects of housing condition on water intake and total fluid intake

The water intake and total fluid intake during the 12 sessions of alcohol access are shown in Supplementary Fig. 1. There was a main effect of sex and session, as well as an interaction between sex and session on water intake (Supplementary Table 1). With regard to housing condition, only minor differences between pair-housed and individually housed males were found – lower water intake in pair-housed males on sessions 2 and 7 (Supplementary Fig. 1A). In female rats, no effect of housing condition on water intake was found (Supplementary Fig. 1B). The average weekly water intake during the 4 weeks of alcohol access is shown in Supplementary Table 3. No differences between pair-housed and individually housed rats were found either in males or in females. With regard to total fluid intake, no differences between pair-housed and individually housed rats were found either in males (Supplementary Fig. 1C) or in females, with the exception of the first session (Supplementary Fig. 1D).

Effects of estrus cycle on female drinking behavior

Vaginal smears were collected Monday through Friday during the four intake weeks in order to track the estrous cycle. On alcohol-access days, the smears were collected prior to alcohol access and the phase on that day was related to the intake of the following 24 hours. The intake data during the different phases of the estrus cycle in pair-housed and individually housed females is shown in Supplementary Fig. 2. No differences between pair-housed and individually housed females were found for any data. No within-group differences were found for alcohol intake (Supplementary Fig. 2A), alcohol preference (Supplementary Fig. 2B), or total fluid intake (Supplementary Fig. 2D). With regard to water intake, pair-housed females had a lower water intake in proestrus relative to diestrus, and individually housed rats had a lower water intake in proestrus and estrus relative to diestrus (Supplementary Fig. 2C).

Effects of social rank on drinking behavior

The effects of social rank based on behavior in the tube test and based on body weight on drinking behavior in pair-housed males is shown in Supplementary Figs. 3 and 4, respectively. No difference between dominant and subordinate males was revealed for any measure either using the tube test or using body weight for assessment of social rank. A consistent partitioning as dominant or subordinate using both the tube test and body weight assessment was found for 40% of the males.

The tube test was not useful for determining social rank in female rats, as in all cases both females remained together in the tube, and therefore no information about dominant and subordinate individuals could be obtained. The effects of social rank based on body weight on drinking behavior in pair-housed females is shown in Supplementary Fig. 5. No difference between dominant

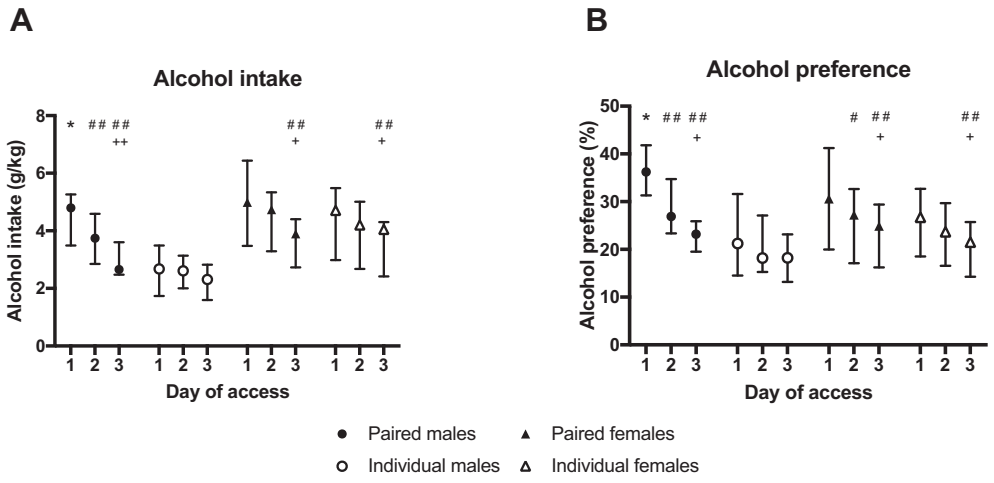

Fig. 5. Average alcohol intake (g/kg; **A**) and preference (%; **B**) across weeks 2–4 in male and female rats on the three consecutive days of access, i.e., Tuesdays, Wednesdays, and Thursdays. Pair-housed rats were separated using a divider during the alcohol intake sessions in order to retrieve individual intake measurements, and individually housed rats were single-housed throughout the 4 weeks. Data are presented as median and interquartile range (n = 10/group). *$p < 0.05$ compared to individually housed animals within the respective sex (Mann–Whitney U test), #$p < 0.05$, ##$p < 0.01$ compared to the intake on the first day of access within the respective group, +$p < 0.05$, ++$p < 0.01$ compared to the intake on the second day of access within the respective group (Wilcoxon's matched pairs test).

and subordinate females was revealed when using body weight for assessment of social rank.

Discussion

The influence of two different housing conditions on voluntary alcohol intake was investigated in adult male and female Wistar rats. Using the modified intermittent access paradigm with a free choice between 20% alcohol and water for three consecutive days and four days of water access (Lundberg et al., 2017; Momeni et al., 2015; Momeni & Roman, 2014; Tjernstrom & Roman, 2018), pair-housed rats were able to spend four out of seven days in full social contact with a conspecific, but were separated during the three consecutive 24-hour alcohol intake sessions when a mesh divider was placed in the cage. Voluntary alcohol intake and preference in the pair-housed rats were compared to that of individually housed rats. The results demonstrate that the pair-housing condition had no impact on voluntary alcohol intake or preference in female rats, compared to individual housing. In contrast, pair-housed males displayed higher voluntary alcohol intake and preference compared to individually housed males during the first 3 weeks, after which this effect ceased and was no longer present during the last sessions and the fourth week.

In recent years, the use of a mesh divider has been introduced as an alternative to single housing for studies of voluntary alcohol intake in rats (Ryabinin & Walcott, 2018). Often the dividers have been used to minimize the impact of individual housing of adolescent rats during alcohol intake sessions (Fernandez et al., 2017; Vetter-O'Hagen et al., 2009; Wille-Bille et al., 2017), but not for comparison with individually housed rats. However, when the same dividers were used in studies of adolescent male Wistar rats during intermittent 2-hour alcohol intake sessions, no difference in alcohol intake and preference was found between pair-housed and individually housed adolescent male rats (Palm & Nylander, 2014). This latter result contrasts with the results found in the present study of adult male rats, but extends the knowledge on the use of a mesh divider in studies of voluntary alcohol intake in adult female rats.

Evidence from preclinical research demonstrates that the social environment can have an impact on voluntary alcohol intake (Anacker & Ryabinin, 2010; Ryabinin & Walcott, 2018). Several studies report higher voluntary alcohol intake in individually housed male rats than in males housed under various social conditions (Deatherage, 1972; Ellison, Daniel, & Zoraster, 1979; Parker & Radow, 1974; Roske, Baeger, Frenzel, & Oehme, 1994; Wolffgramm, 1990). However, there are also reports demonstrating that housing condition had no impact on alcohol intake in male and female rats (Hannon & Bolter, 1980), while another study found that adult male and female rats consumed more sweetened alcohol under social circumstances than when individually housed (Varlinskaya, Truxell, & Spear, 2015).

The present results indicate that male rats initially were more sensitive to the pair-housing condition than females. One explanation for the differences obtained could be related to the degree and form of social interaction with conspecifics. Female rats do not form strong dominance relationships (Haller, Fuchs, Halasz, & Makara, 1999), in contrast to male rats (Barker, George, Howarth, & Whittaker, 2017). Thus, the repeated social interactions and separations, caused by the divider, may induce initial social stress in males, which could explain the higher alcohol intake and preference in males during the first weeks relative to individually housed rats. In support of this is a study showing that social disruption increased alcohol intake in male rats (Ellison et al., 1979). A limitation in the present study is that no measurements of glucocorticoid levels were obtained in male and female rats in the different housing conditions.

In our earlier studies in male rats (Momeni et al., 2015; Momeni & Roman, 2014), we demonstrated that the modified intermittent access paradigm gives rise to a weekly alcohol deprivation effect (Sinclair & Senter, 1967; Vengeliene, Bilbao, & Spanagel, 2014), with higher alcohol intake on the first day of access relative to the following two days. This pattern was apparent in the pair-housed males in agreement with earlier studies (Tjernstrom & Roman, 2018) and also present in the female rats, similar across housing conditions. In contrast to previous findings (Momeni et al., 2015; Momeni & Roman, 2014), the alcohol deprivation effect was not found in individually housed males. However, using the conventional intermittent access paradigm with alcohol available on Mondays, Wednesdays, and Fridays (Carnicella et al., 2014), a higher intake on Mondays has been observed only in adolescent rats but not in adult males or females (Schramm-Sapyta et al., 2014). Notably, in the pair-housed males, the evident alcohol deprivation effect may be driving the effect of housing condition on alcohol intake and preference observed during the first 3 weeks.

The outbred Wistar rats from Envigo used in the current study have repeatedly demonstrated a higher voluntary alcohol intake in male rats in various home cage drinking paradigms compared to Wistar substrains from other vendors (Goepfrich, Gluch, Friemel, & Schneider, 2013; Momeni et al., 2015; Palm et al., 2011; Wood et al., 2017), while this result is less pronounced in female rats (Lundberg et al., 2017). In the present study, female rats had a higher alcohol intake than males independent of housing condition, which agrees with the literature using both outbred strains and selectively bred lines of rats (Becker & Koob, 2016; Priddy et al., 2017), also using the intermittent access paradigm in Wistar rats (Priddy et al., 2017). Strikingly, few preclinical studies on voluntary alcohol intake have directly compared males and females (Becker & Koob, 2016), but see, for example Priddy et al., 2017; Schramm-Sapyta et al., 2014; and Varlinskaya et al., 2015. One reason may relate to concerns about variability related to the female hormonal cycle. A recent meta-analysis demonstrated that female rats are no more variable than males on a variety of measurements (Becker, Prendergast, & Liang, 2016). The lack of influence of the estrus cycle on alcohol intake and preference revealed in the current study is in agreement with previous studies using the intermittent access paradigm in Wistar rats (Priddy et al., 2017), and is consistent with other studies using outbred and selectively bred rats in continuous access paradigms (e.g., Ford, Eldridge, & Samson, 2002; Priddy et al., 2017; Roman et al., 2005).

Results from this study indicate that social rank in pair-housed males had no effect on voluntary alcohol intake and preference, neither when the tube test nor when body weight was used. However, the results should be viewed as preliminary, considering the low number of animals in each group and the fact that the tube test has received criticism (Miczek & Barry, 1975). Moreover, different approaches for assessment of social rank do not always produce consistent results (Benton, Dalrymple-Alford, & Brain, 1980), which may explain the different outcomes using the tube test and body weight assessment for partitioning male rats into dominant and subordinate, respectively. The fact that female rats do not form strong dominance relationships (Haller et al., 1999) may explain why neither the tube test nor body weight was useful for assessing social rank in females.

In conclusion, this study demonstrates an alternative housing condition with few long-term influences on voluntary alcohol intake, especially evident in female rats. Therefore, with further studies, the use of a mesh divider may be able to limit single housing and thereby refine housing conditions in limited or intermittent access alcohol consumption paradigms, which today

represent the most commonly used paradigms to study voluntary alcohol in the home cage in rats.

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Author statement

The authors declare that the study was conducted in the absence of conflicting interests. The data will be made available on request.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.alcohol.2019.12.005.

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