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ORIGINAL INVESTIGATION

Effects of social housing conditions on ethanol-induced behavioral sensitization in Swiss mice

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Abstract

Rationale In previous animal model studies, it was shown that drug sensitization is dependent upon physical environmental conditions. However, the effects of social housing conditions on drug sensitization is much less known.

Objective The aim of the present study was to investigate the effects of social conditions, through the size of housing groups, on ethanol stimulant effects and ethanol-induced behavioral sensitization in mice.

Materials and methods Male and female Swiss mice were housed in groups of different sizes (isolated mice, two mice per cage, four mice per cage and eight mice per cage) during a six-week period. A standard paradigm of ethanol-induced locomotor sensitization was then started with one daily injection of 2.5 g/kg ethanol for 8 consecutive days.

Results The results show that social housing conditions affect the acute stimulant effects of ethanol. The highest stimulant effects were observed in socially isolated mice and then gradually decreased as the size of the group increased. Although the rate of ethanol sensitization did not differ between groups, the ultimate sensitized levels of ethanol-induced stimulant effects were significantly reduced in mice housed in groups of eight.

Conclusions These results are consistent with the idea that higher levels of acute and sensitized ethanol stimulant effects are observed in mice housed in stressful housing conditions, such as social isolation.

Keywords Ethanol · Behavioral sensitization · Locomotor activity · Environmental enrichment · Social enrichment – social isolation · Mice

Introduction

The development of drug and alcohol addiction is highly dependent upon physical and social environmental conditions. In particular, stressful living conditions have been shown to promote drug and alcohol abuse in humans (Antelman et al. 1980; Kalivas and Stewart 1991; Keyes et al. 2012; Koob and Volkow 2016; Sinha 2009; Wojdala et al. 2020). Indeed, coping with stress is reported to be an important motive for drug and alcohol consumption in various populations (Edwards et al. 1981; Khantzian 2013; Lazarus 1999). This is believed to explain in part the high comorbidity between post-traumatic stress disorder and substance use disorder (Davis et al. 2013; Matonda-Ma-Nzuzi et al. 2019; Sofuoglu et al. 2014). However, epidemiological studies have also shown that drinkers with high levels of alcohol consumption are more vulnerable to stress (Keyes et al. 2012; Sayette 1999; Sinha 2008). This suggests that stress and substance use disorders might be linked by more complex, bi-directional, causal relationships. In that respect, animal models of substance and alcohol use can help to detangle the relationships between stress and drug abuse. Among the various forms of chronic stress, the impoverishment of the physical and social housing environment in laboratory animals has attracted a great deal of attention in recent years. For instance, many recent studies reported that environmental enrichment of housing conditions reduces the negative effects of stress in rodents (Akre et al. 2011; Bahi 2017; Bahi and Dreyer 2020; Bailoo et al., 2018; Camarini et al. 2018; Olsson & Dahlborn, 2002).

Environmental enrichment of housing (EE) is generally defined as any modification aimed at improving the physiological and psychological well-being of captive animals by

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providing them with stimuli bound to the specific needs of their own species (Baumans 2005; Baumans and Van Loo 2013; Fraser 2009; Newberry 1995; Sztainberg and Chen 2010). These enriched environmental modifications generally pertain to several properties intrinsic to the animal species, such as the psychomotor properties (e.g. running wheel), the social properties (e.g. housing in social groups), the visuospatial properties (e.g. providing handling objects), the cognitive properties (e.g. labyrinths or tunnels) or the nutritional properties (e.g. foraging or food variation). In many studies, environmental enrichment consists in housing captive animals in social groups, while providing them with a variety of objects, which could facilitate sensory, cognitive, motor and social stimulation compared to the usual "standard housing conditions". Nowadays, "standard housing conditions" for experimental animal have widely evolved. According to the directives and guidelines for the health and protection of animals used for scientific purposes (e.g. ARRIVE or the 3 R's rule), housing of mice and rats have to be refined at least with nesting materials and as much as possible in a physically and socially appropriate enriched environment. Such enriched environment leads to important beneficial consequences both for their welfare and the quality of research data derived from them (Bailoo et al., 2018; Olsson & Dahlborn, 2002; Olsson & Westlund, 2007). In contrast, old standard housing conditions for mice and rats (bedding, food and water) were designed primarily for economic (minimal use of space, equipment and labor), ergonomic (ease of handling, visibility of animals), hygiene (easy to sanitize) and standardization (minimization of variation) reasons (Bailoo et al., 2018; Olsson et al. 2003). However, these old-fashioned standard conditions, and especially social isolation, are now unacceptable and considered as an environmental impoverishment relative to the needs of the species for both rats and mice.

EE in rodents is widely used in the scientific literature as an animal model for studying the effect of the physical, cognitive, and social environment on the development of various forms of psychopathology and neurological diseases. Appropriate enriched environmental conditions promote adapted social behaviors in experimental animals and reduce abnormal anxiety reactions and depressive symptoms. Kulesskaya et al. (2011) reported that housing conditions had a significant impact on various mice behaviors. For example, female mice housed in enriched conditions displayed faster and better-quality learning, as well as an increase in exploratory behaviors and reduced anxious behaviors. EE was also shown to induce morphological, molecular and neurochemical changes in different brain areas (Diamond 2001; Landers et al. 2011; Rosenzweig and Bennett 1972; Sale et al. 2009). For example, EE was shown to alter brain plasticity and to promote dendritic ramifications in several brain areas (Greenough et al. 1973; Kempermann 2019; Kolb et al. 2003; Komleva et al. 2013). In addition, in-vivo MRI studies reported that both male mice and rats housed in social groups with enriched physical conditions expressed neuroanatomical beneficial changes such as volumetric cerebral changes in the brain areas devoted to higher cognitive processes (Scholz et al. 2015; Susser and Wallace 1982).

In the field of animal models of drug addiction, EE was also reported to be a reliable protective or therapeutic agent against the effetcs of addictive drugs. For example, many studies reported that various forms of EE have beneficial consequences against cocaine effects. EE was shown to reduce the acquisition, expression and reinstatement of cocaine self-administration (Cosgrove et al. 2002; Lynch et al. 2010; Powell et al. 2020; Smith et al. 2008; Zlebnik et al. 2010) to prevent concaine-induced conditioned place preference (Chauvet et al. 2009; Freese et al. 2018; Solinas et al. 2008) and behavioral sensitization (Chauvet et al. 2009; Geuzaine and Tirelli 2014; Lespine and Tirelli 2015, 2018; Solinas et al. 2008) in both male and female rodents. Similar results were obtained with other drugs of abuse, such as amphetamines (Garcia et al. 2017; Stairs et al. 2017), nicotine (Hamilton et al. 2014; Mesa-Gresa et al. 2013; Sikora et al. 2018) and heroin (Barrera et al. 2021; Galaj et al. 2016; Imperio et al. 2018). Regarding alcohol, EE was recently reported to decrease alcohol consumption and preference in both male and female rodents (Berardo et al. 2016; Deehan et al. 2011; Holgate et al. 2017; Lopez and Laber 2015; Marianno et al. 2017; McCool and Chappell 2009; Munn et al. 2011). Whereas many studies have investigated the effects of physical EE in animal models of drug addiction, much less is known about the effects of social enrichment of the housing conditions. Social isolation is well established as a stress condition in mice and rats from both sexes (Araujo et al. 2005; Conrad and Winder 2011; Deehan et al. 2007; Gamallo et al. 1986; Lopez and Laber 2015; Olsson & Westlund, 2007; Valzelli 1973). However, the optimal size of housing groups of mice and rats is unclear, as overcrowded cages may also become stressful living conditions (Delaroque et al. 2021; Lin et al. 2015). In mice, according to published guidelines like ARRP (Animal Research Review Panel) and literature reviews, the optimal size for a group of adult mice is three to five for females and three to four for males (Kappel et al., 2017; Olsson & Westlund, 2007; Schuhr 1987; Van Loo et al. 2001a). As social conditions probably account for a significant part of EE, further studies are required to investigate their effects in animal models of drug addiction.

The aim of the present study was to investigate the effects of social conditions, through the size of housing groups, on ethanol stimulant effects and ethanol-induced behavioral sensitization in Swiss mice. Drug sensitization is believed to result from brain central mechanisms and is suggested to play a critical role in ethanol and drug addiction (Vanderschuren and Pierce 2010). For example, the incentive sensitization theory (Robinson and Berridge 1993) is one of the current leading theories of addiction. The theory places the mechanism of dopamine sensitization at the heart of its explanation of drug-induced behavioral dependence. In rodent studies, drug-induced behavioral sensitization is considered homologous to the intensification of drug craving after repeated episodes of drug consumption and the paradigm is therefore used to model various features of drug addiction. In laboratory rodents, ethanol-induced behavioral sensitization is most often tested by the progressive increase in its locomotor stimulant effects over repeated administrations of the same ethanol dose (Didone et al. 2008, 2019; Masur et al. 1986; Masur and Boerngen 1980). Previous studies reported that the locomotor stimulant effects of ethanol are affected by social housing conditions. For example, Päivärinta (1990) showed that socially isolated male mice are more vulnerable to the locomotor stimulant effects of a single ethanol dose. Araujo et al. (2005) also reported higher levels of ethanol-induced locomotor sensitization in female mice from both isolation and overcrowded cages. In the present study, male and female Swiss mice were housed in groups of different sizes (isolated mice, two mice per cage, four mice per cage and eight mice per cage) during a six-week period starting on post-natal day 28. However, and in contrast with the study from Araujo et al. (2005), the sizes of the cages were adapted to avoid stressful overcrowding conditions. A standard paradigm of ethanol-induced locomotor sensitization was then started with one daily injection of 2.5 g/kg ethanol during 8 consecutive days. Based on previously published studies (see Araujo et al. 2005; Päivärinta 1990) and the results of previous pilot studies in our laboratory (Didone, van Ingelgom and Quertemont, unpublished results), it was hypothesized that isolation-housed mice would be more vulnerable to the stimulant effects of ethanol. In contrast, mice housed in groups, and especially in groups of four or eight, should display a reduced sensitivity to the stimulant effects of ethanol and their sensitization upon repeated administrations.

Materials and methods

Subjects

For the present study, 128 female Swiss mice were bred in our colony with male and female progenitors purchased from Janvier Laboratories (Le Genet-Saint-Isle, France). Pups were first gathered with their mothers in groups of three litters in wide breeding cages from birth to weaning (post-natal days 0 to 21) and then housed in groups of 8 from postweaning to the beginning of adolescence (post-natal days 21 to 28) in smaller breeding cages. After this breeding period, 128 four-weeks old female mice were randomly allocated into four groups with different housing conditions: single housed mice (G1), 2 mice per cage (G2), 4 mice per cage (G4) and 8 mice per cage (G8), each group of mice including 32 mice. Mice from G1 and G2 groups were housed in standard cages (33.1 × 15.9 × 13.2 cm, floor area of 335 cm^2), mice from the G4 group were housed in medium cages (36.5 × 20.7 × 14 cm, floor area of 530 cm^2) and mice from the G8 group in large cages (42.5 × 27.6 × 15.3 cm, floor area of 820 cm^2). All cages were made of transparent polycarbonate (Tecniplast, Milano, Italy), filled with pine sawdust bedding, and arranged on shelves allowing olfactory, visual and acoustic interactions. The animal room was maintained on a 12-hour light/dark cycle (lights on at 7:00 A.M.) with low light intensity levels (50–60 lx), kept at a temperature of 19–24 °C and a relative humidity at 40 to 50%. Standard diet food (Carfil Quality BVDA, Oud-Turnhout, Belgium) and tap water were available ad libitum except during the experimental procedures. All experimental procedures were conducted during the light phase of the cycle, between 7:00 A.M. and 1:00 P.M and according to the Belgian implementation of the animal welfare guidelines laid down by the European Union ("Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes"). This protocol was reviewed and approved by the Ethics and Animal Care Committee for the use of experimental animals from the University of Liège. Every effort was made to minimize the number of animals used and their suffering. A second part of the study was initially planned with male mice using the same methodology and an identical number of animals. However, the experiment with males had to be interrupted after a first experimental cohort of 64 mice due to the levels of injuries observed in some of them. Four male mice had to be euthanized and four others had to receive care due to leg injuries before the end of the sensitization protocol. Consequently, it was decided to end the male part of the experiment. The results of this first cohort are reported as supplementary material.

Experimental housing conditions

The four experimental housing conditions were defined by the presence or absence of congeners into the home cage. Mice of the G1 group were single housed (isolated), whereas mice of the G2 (paired), G4 (standard group) and G8 (large group) groups were housed respectively in groups of 2, 4 or 8 animals. These housing conditions remained constant during the whole experiment from the pre-testing phase (6 weeks) to the experimental phase (10 days). Except for the number of mice per cage, all other housing and cleaning conditions were identical between groups.

Drugs

In the present study, ethanol injections were 20% v/v, diluted from 99.9% ethanol in an isotonic sterile 0.9% saline solution. Ethanol and control saline solutions were injected via the intraperitoneal (i.p.) route. The 2.5 g/kg ethanol dose was selected on the basis of previous studies showing an optimal development of ethanol sensitization at this dose in Swiss mice (Didone et al. 2008, 2016, 2019; Legastelois et al. 2014; Quoilin et al. 2014).

Behavioral test chambers

Locomotor activity was recorded using a videotracking software (Viewpoint, Lyon, France). The testing environment is composed of two sets of four squared open fields (40 × 40 × 40 cm), allowing the recording of eight mice simultaneously. These sets form an 80 × 80 cm squared complex. The floor and the wall are made in black hard plastic (Forex - View Point, Lyon, France), contrasting the background of the environment with the color of the mouse. A camera (Bosch - Viewpoint, Lyon, France) is positioned directly above the open fields, such that the whole surface is covered. The videotracking software measures the horizontal travel distance (cm) of the mice. Target detection considers the mice center of gravity. The testing environment was cleaned with a disinfectant/water solution between each batch.

Experimental design and procedure

The whole experiment lasted 52 days, including 42 days (6 weeks) for the pre-testing phase and 10 days for the ethanol sensitization and testing procedure. This experimental protocol (Fig. 1) is derived and adapted from Didone et al. (2008, 2016, 2019) and Broadbent et al. (2005), including a habituation session to the testing environment, one daily ethanol or saline challenge for 8 sessions (sensitization induction) and a test session for the expression of the sensitized effects (sensitization expression). During the 6-weeks pre-testing phase, mice were maintained in their specific housing conditions. The sensitization protocol started on the first day of the 7th week with the habituation session. All mice were moved to the experimental room, weighed, and injected with sterile 0.9% saline and their locomotor activity was immediately recorded for the next 5 min (distance travelled in cm). In each housing condition group, mice were then subdivided into two experimental subgroups, which were equated for basal locomotor activity during the habituation session (Fig. 1). Together, all these groups constituted a 4 (housing conditions) x 2 (pharmacological treatments) experimental design.

The induction of sensitization started the next morning after the habituation session. For 8 days, mice were daily injected with 2.5 g/kg ethanol (or saline for the control groups), immediately placed into the open fields and their

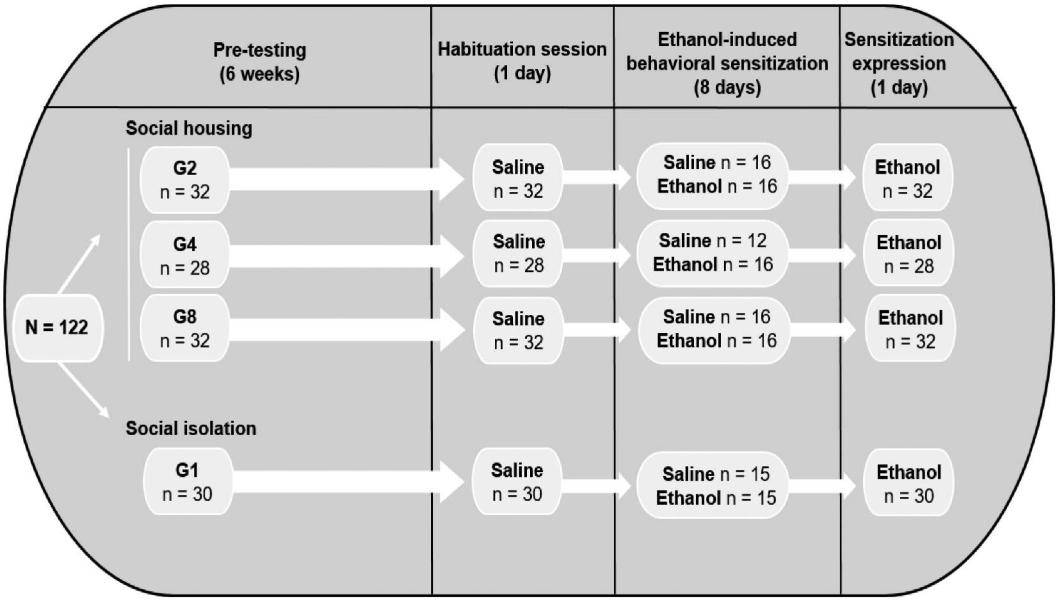

Fig. 1 Experimental design of the study. Female Swiss mice were randomly divided in four groups according to the housing conditions: single housed (G1), in pairs (G2) or in groups of 4 (G4) and 8 (G8) animals

locomotor activity was recorded for the next 5 min. This session duration was chosen in order to specifically capture the stimulant effects occurring during the ascending limb of the blood alcohol concentrations (Didone et al. 2008, 2019; Quoilin et al. 2012a). Mice were weighed again on day 1 and day 5 to check for body weight fluctuations.

The morning after the last sensitization session, an ethanol test session was carried out. All the mice were weighted and injected with 2.5 g/kg ethanol and immediately placed into the open fields. Their locomotor activity was recorded for 30 min. The aim of this last session was to test for intergroup ethanol sensitization by comparing mice of the saline control groups receiving their first ethanol challenge with mice of the ethanol groups receiving their 9th daily ethanol challenge. Relative to the sensitization sessions, the test session was of longer duration in order to capture possible delayed effects in the time course of ethanol-induced locomotor effects (Didone et al. 2008, 2019).

Attrition

During the pre-testing phase and before the start of the ethanol sensitization procedure, six mice had to be removed from the experimental protocol. Two mice of the G1 group died from unknown causes. Additionally, a male mouse was assigned by mistake into a cage of the G4 group, such that the three female mice in the cage were found pregnant; those were therefore removed from the experiment.

Statistical power analyses

Before the start of the study, minimum sample sizes for the experimental groups were estimated with a priori statistical power analyses on the main analysis of the sensitization development. Power was set at 0.8, type-I error rate at 0.05 and an effect size of $f = 0.125$. This analysis was performed with GPower 3.1.

Data analysis

All behavioral data were analyzed using fixed-effect or mixed-effect analyses of variance (ANOVA) followed by Newman–Keuls post hoc tests or planned contrasts. In case of significant Levene test, square-root transformations normalized raw data prior to the ANOVA more nearly meeting the assumption of homogeneity of variance. Effect sizes (standard or partial eta squared and Cohen's d) with 95% confidence intervals (CI) were calculated for the statistically significant effects. Statistical significance was set at $p < 0.05$. All analyzes and charts were performed with RStudio 2022.12.0 + 353 (ggplot2) and Statistica 13.2.

Locomotor activity on the habituation session was analyzed using a fixed-effect one-way ANOVA. For the first ethanol session (acute response), locomotor activity was analyzed using a fixed-effect two-way (pharmacological treatment x housing condition) ANOVA. The development of ethanol sensitization across the 8-ethanol sessions was analyzed using a mixed-effect three-way ANOVA with pharmacological treatments and housing conditions as between-subject factors and the 8-sensitization sessions as a within-subject factor. Following a significant Mauchly's test, the Greenhouse-Geisser correction was applied to adjust for violations of the assumptions of compound symmetry and sphericity. In order to test for within-group ethanol sensitization, planned contrasts were performed independently for each experimental subgroup to compare their mean locomotor activity on the first and last sensitization session. Planned contrasts were also computed for ethanol subgroups to compare the last sensitization session between the four groups, i.e. to test for differences in ethanol-sensitized responses. Finally, the results of the sensitization test session were analyzed with a fixed-effect two-way (pharmacological treatments x housing conditions) ANOVA.

Results

The results for the male cohorts are reported as supplementary materials.

Habituation session

The one-way ANOVA performed on the locomotor activity during the five minutes of the habituation session displayed a significant main effect of the housing conditions [$F(3,118) = 8.08$; $p < 0.001$; $\eta^2 = 0.17$ with 95% CI (0.06, η^2, 0.28)]. As shown in Fig. 2A, the Newman–Keuls posthoc comparisons revealed lower levels of mean locomotor activity in mice housed in groups of eight (G8) relative to all other groups ($p < 0.001$): vs. G1: $d = 0.95$ with 95% CI (0.42, d, 1.48), vs. G2: $d = 0.96$ with 95% CI (0.44, d, 1.48) and vs. G4: $d = 0.87$ with 95% CI (0.34, d, 1.40).

Acute session

The two-way ANOVA computed on the locomotor activity during the 5 min of the first sensitization session displayed a significant main effect of housing conditions [$F(3,113) = 7.60$; $p < 0.001$; $\eta^2_p = 0.17$ with 95% CI (0.05, η^2_p, 0.27)] and a significant housing condition x pharmacological treatment interaction [$F(3,113) = 3.19$; $p = 0.026$ $\eta^2_p = 0.08$ with 95% CI (0, η^2_p, 0.16)], whereas the main effect of the pharmacological treatment was not statistically

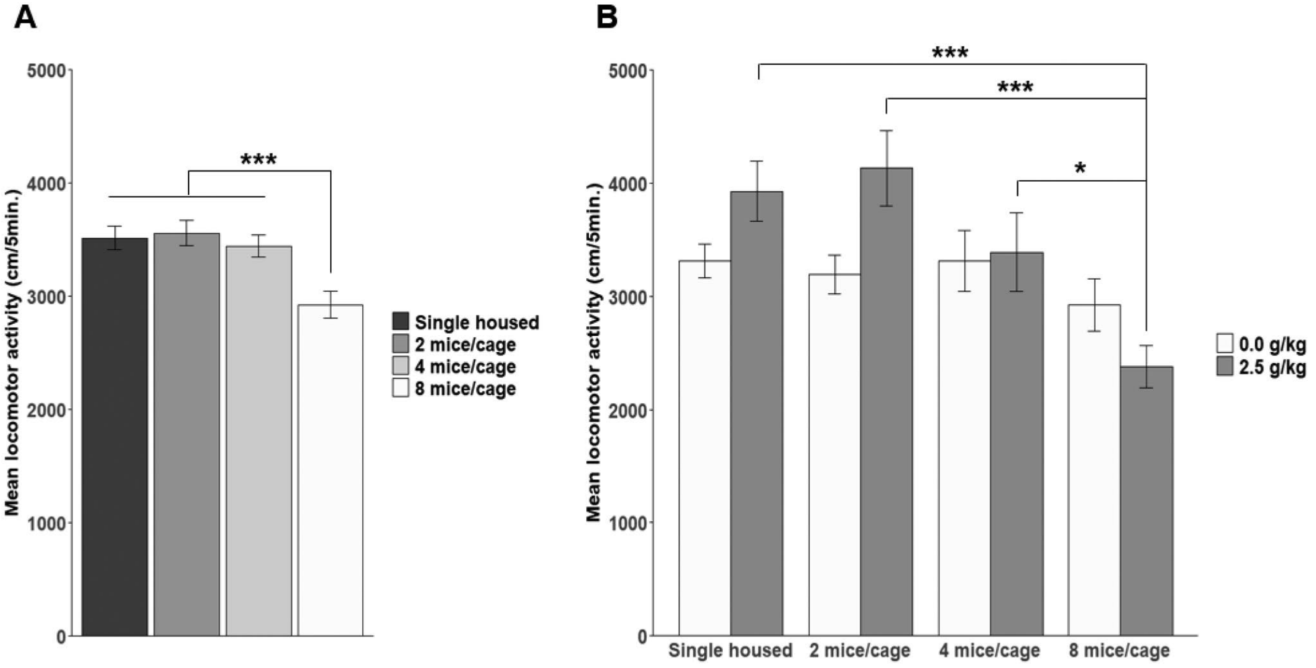

Fig. 2 **A.** Locomotor activity (Mean ± SEM) on the habituation session after a saline challenge.*** $p < 0.001$: significantly different from the G8 ethanol group.**B.** Locomotor activity (Mean ± SEM) on the acute session after the injection of 2.5 g/kg ethanol or 0.0 g/kg (saline).*** $p < 0.001$ and * $p < 0.05$: significantly different from the G8 ethanol group

significant [F(1,113) = 1.20; $p = 0.275$; η^2_p=0.01]. The Newman–Keuls post-hoc comparisons revealed that ethanol-injected mice in the G8 group displayed reduced locomotor activity relative to ethanol-injected mice in G4 [$p = 0.037$; d = 0.90 with 95% CI (0.09, d, 1.71)], G2 [$p < 0.001$; d = 1.73 with 95% CI (0.90, d, 2.56)] and G1 [$p < 0.001$; d = 1.63 with 95% CI (0.82, d, 2.44)]. Relative to their respective saline control groups, there were statistically significant acute stimulant effects in the G1 and G2 ethanol groups, but not in the G4 and G8 ethanol groups. The results of one mouse in the G2/saline group were lost due to technical recording issues. The results are shown in Fig. 2B.

Ethanol-induced behavioral sensitization

The mixed-design 2 × 4 × 8 ANOVA computed on the locomotor activity during the sensitization sessions showed a significant main effect of housing conditions [F(3,112) = 8.09; $p < 0.001$; η^2_p=0.18 with 95% CI (0.06, η^2_p, 0.28)], pharmacological treatment [F(1,112) = 89.51; $p < 0.001$; η^2_p=0.44 with 95% CI (0.31, η^2_p, 0.54)] and sensitization sessions [F(7,784) = 3.75; $p < 0.001$; η^2_p=0.03 with 95% CI (0.01, a η^2_p, 0.05)], remaining significant at $p < 0.001$ after G-G. adjustment, and a significant interaction between sensitization sessions and pharmacological treatment [F(7,784) = 34.57; $p < 0.001$; η^2_p=0.24 with 95% CI (0.18, a η^2_p, 0.28)], remaining significant at $p < 0.001$ after G-G. adjustment. This significant main interaction indicated an overall development of sensitization in all groups of mice following repeated ethanol administrations. The planned contrasts showed significant differences between the last and the first sensitization session in mice repeatedly injected with ethanol in each housing group (G1: [F(1,112) = 23.40; $p < 0.001$; d = 1.56 with 95% CI (0.75, d, 2.37)], G2: [F(1,112) = 10.50; $p < 0.01$; d = 0.67 with 95% CI (0.04, d, 1.38)], G4: [F(1,112) = 21.52; $p < 0.001$; d = 0.99 with 95% CI (0.26, d, 1.72)] and G8: [F(1,112) = 33.07; $p < 0.001$; d = 1.44 with 95% CI (0.66, d, 2.22)]). The planned contrasts performed on the locomotor activity of ethanol mice during the last sensitization session also revealed that G8 ethanol mice displayed lower levels of locomotor activity than G2 [F(1,112) = 10.05; $p < 0.01$; d = 0.83 with 95% CI (0.11, d, 1.55)]) and G1 [F(1,112) = 16.12; $p < 0.001$; d = 1.40 with 95% CI (0.62, d, 2.18)] ethanol mice. In other words, the sensitized levels of locomotor activity reached by ethanol G8 mice on the last sensitization session was significantly reduced relative to these two housing groups, whereas the rate of development of ethanol sensitization was not significantly different from all the other groups. Figure 3A shows the locomotor activity of mice from the four housing conditions groups on every sensitization session and Fig. 3B highlights the locomotor sensitized levels on the last sensitization session.

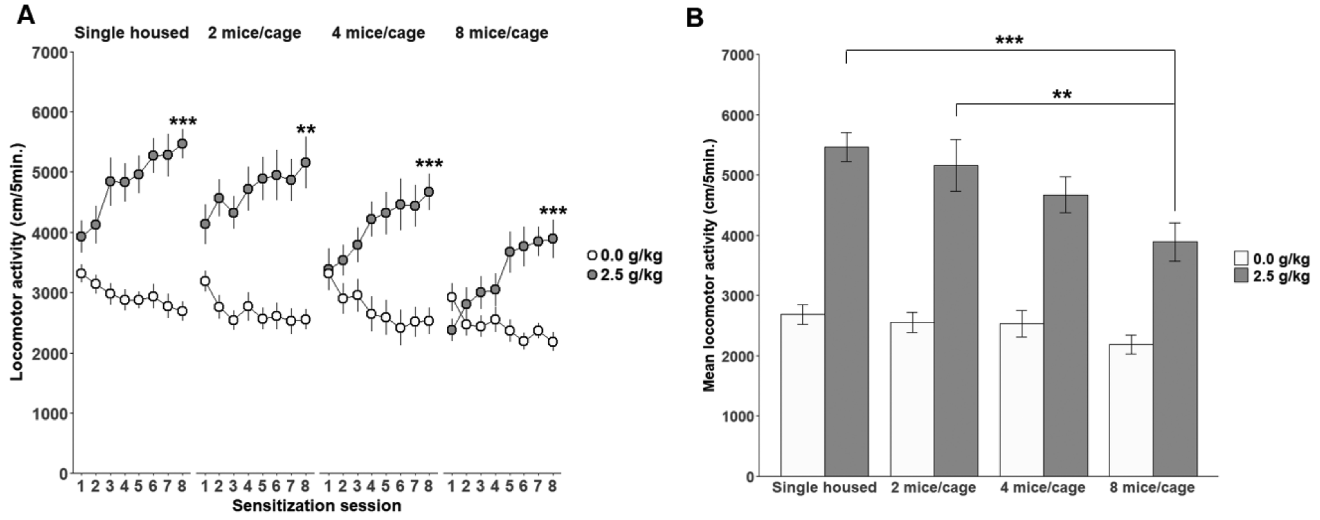

Fig. 3 **A.** Development of ethanol sensitization after eight injections of either 2.5 g/kg ethanol or 0.0 g/kg (saline) for the four groups of mice (Mean ± SEM).*** $p < 0.001$ and ** $p < 0.01$: significantly different from the first session, as indicated by the planned contrasts.**B.** Sensitized levels of locomotor activity on the last sensitization session (8th session) for the four groups of mice (Mean ± SEM).*** $p < 0.001$ and ** $p < 0.01$: significantly different from the G8 ethanol group

Sensitization test session

The two-way ANOVA computed on the locomotor activity during the 30 min sensitization test session (with a 2.5 g/kg ethanol challenge) revealed a main effect of housing conditions [F(3,112) = 7.06; $p < 0.001$; η^2_p=0.16 with 95% CI (0.04, η^2_p, 0.26)] and treatment [F(1,112) = 60.80; $p < 0.001$; η^2_p=0.35 with 95% CI (0.21, η^2_p, 0.47)]. However, the interaction between these two factors was statistically non-significant [F(3,112) = 0.62; $p = 0.60$; η^2_p=0.02]. The Newman–Keuls post-hoc comparisons revealed that all mice from the four housing conditions significantly expressed the sensitized effects of ethanol. In other words, all the ethanol groups displayed more locomotor activity than all their respective control saline groups receiving their first ethanol injection [G1: $p < 0.05$; d = 0.83 with 95% CI (0.08, d, 1.58)], [G2: $p < 0.01$; d = 1.40 with 95% CI (0.63, d, 2.17)], [G4: $p < 0.001$; d = 1.39 with 95% CI (0.57, d, 2.21)] and [G8: $p < 0.001$; d = 1.84 with 95% CI (1.01, d, 2.67)]. Reduced ethanol stimulant effects (both acute and sensitized) in the G8 group relative to all other groups explain the statistically significant main effect of housing conditions. Interestingly, the Newman–Keuls post-hoc comparisons also revealed that G8 mice repeatedly challenged with saline expressed significantly reduced acute ethanol locomotor effects relative to all other groups of saline-treated mice [vs. G1: $p < 0.001$; d = 1.37 with 95% CI (0.64, d, 2.09)], G2: $p < 0.01$; d = 1.30 with 95% CI (0.60, d, 2.01)] and [G4: $p < 0.05$; d = 0.99 with 95% CI (0.23, d, 1.75)]. Figure 4 shows the locomotor activity of mice from the four housing condition groups on the 30 min sensitization test session.

Discussion

The results of the present study show that social housing conditions in female Swiss mice alter the acute stimulant effects of ethanol and the sensitized response to ethanol at the end of a sensitization procedure. More precisely, the highest stimulant responses to ethanol were observed in single housed mice and then gradually decreased as the size of the group increased to reach statistical significance in mice housed in groups of eight. Although the rate of ethanol sensitization did not differ between groups, the ultimate sensitized levels of ethanol-induced stimulant effects after the 8th injection were significantly reduced in mice housed in groups of eight. It is also noteworthy that mice of the control group receiving their first ethanol injection on the test session also displayed significantly reduced levels of acute ethanol stimulant effects when housed in groups (see control group of Fig. 4). Finally, mice housed in groups of eight also showed reduced levels of locomotor activity on the first habituation session. Very similar results were obtained in male mice (see supplementary material), although the experiment with males was ended after a first experimental cohort due to injurious aggression between male mice.

In female Swiss mice, the present results are consistent with the idea that higher levels of acute and sensitized ethanol stimulant effects are observed with chronic stressful housing conditions. A number of previous studies have reported higher levels of locomotor stimulant effects for a variety of abused drugs when female rodents are submitted to chronic stress, such as social isolation, overcrowding, early maternal separation or social conflict (Araujo et al. 2005, 2006; Camarini et al. 2018; Fosnocht et al. 2019;

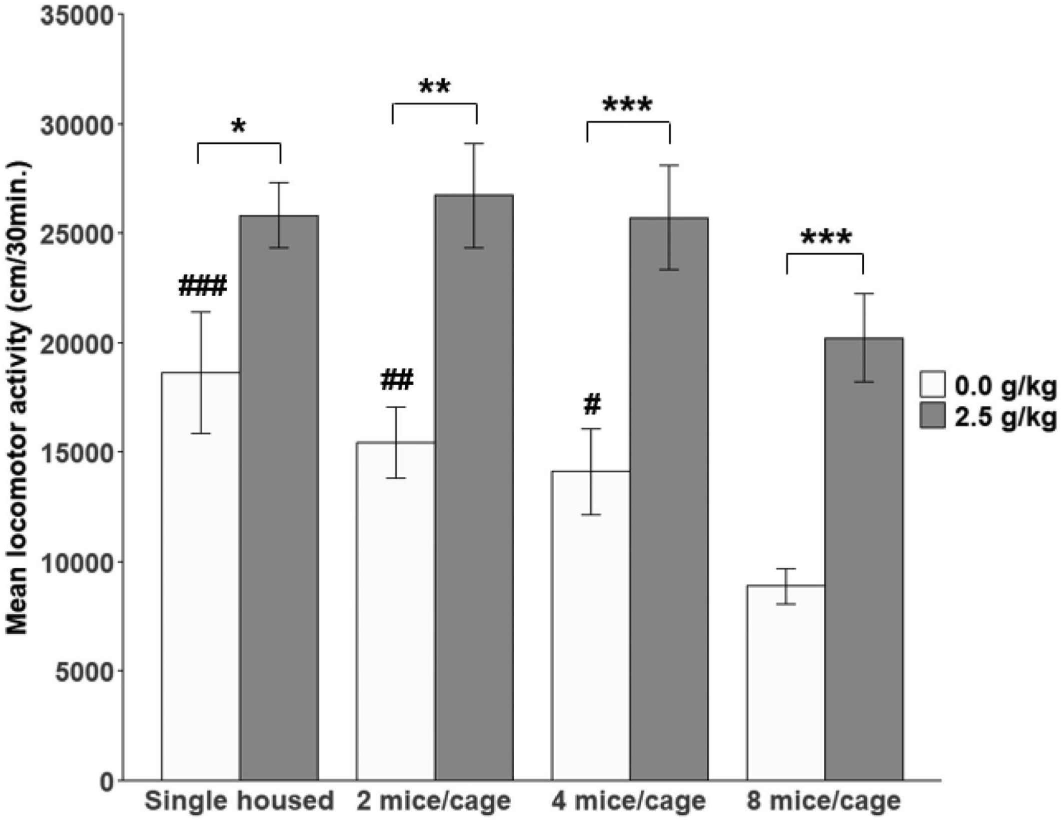

Fig. 4 Locomotor activity on the sensitization test session, the next day after the last sensitization session (9th session). All mice were injected with 2.5 g/kg ethanol.*** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$: significantly different from the respective control group that was repeatedly injected with saline during the sensitization procedure.### $p < 0.001$, ## $p < 0.01$ and #$p < 0.05$: respectively different from G8/saline control group

Gamallo et al. 1986; Kawakami et al. 2007, 2016; Lynch 2006; Sinha 2008). Concomitantly, other studies have shown that housing conditions intended to reduce stress, such as wheel running, physical enrichment or social housing, also decreased the stimulant effects of abused drugs in both males and females (Araujo et al. 2005; Bahi 2017; Cosgrove et al. 2002; Darlington et al. 2016; Lespine and Tirelli 2015, 2018; Lopez and Laber 2015; Rodríguez-Ortega et al. 2018; Rueda et al. 2012; Solinas et al. 2008). In the present study, the highest levels of acute and sensitized ethanol stimulant effects were recorded in female mice housed in social isolation, which is generally considered as a stressful housing condition in mice (Du Preez et al. 2021; Farbstein et al. 2021; Matsumoto et al. 2019; Olsson & Westlund, 2007; Sullens et al. 2021). This is in agreement with the results of Araujo et al. (2005) and Päivärinta (1990) who also showed that socially isolated mice are more vulnerable to the stimulant effects of ethanol. However, in contrast to Araujo et al. (2005), housing female mice in groups of large sizes (8 in the present study) did not lead to new increases in the locomotor stimulant effects of ethanol. In the study from Araujo et al. (2005) the relationship between the size of the housing groups and the levels of ethanol stimulant effects followed a U shape with the highest levels of stimulant effects observed in female isolated mice and in female mice housed in groups of 15. However, and in contrast to that study, the sizes of the cages were adapted in the present study to focus on social conditions and avoid overcrowding from insufficient living space. Therefore, the present results are not in contradiction with those of Araujo et al. (2005) and together are consistent with the thesis that stressful housing conditions, whether it be from social isolation or from overcrowding, increase the locomotor stimulant effects of ethanol in female mice.

In male Swiss mice, the general conclusion is less obvious. The same effects than in female were observed for the relationship between the size of the housing groups and the acute and sensitized ethanol stimulant effects. As in female Swiss mice, the highest levels of ethanol stimulant effects were recorded in isolated male mice, while the lowest levels were observed in male mice housed in groups of eight. However, male mice housed in groups and especially in groups of eight also showed increased levels of aggression, sometimes leading to severe injuries. Previous studies had already shown that male mice housed in groups are highly intolerant to each other, tend to fight and can get hurt, sometimes seriously (Bisazza 1981; Kappel et al., 2017; Marashi et al. 2004; Olsson & Westlund, 2007; Van Oortmerssen 1971). In that context, some strains of mice are more aggressive than others and male Swiss mice are notorious for their high propensity to fight (Kappel et al., 2017; Bisazza 1981). Furthermore, the ethanol sensitization procedure was shown to increase aggression (Fish et al. 2002). This is one of the reasons why we usually favor female mice in our sensitization studies (Didone et al. 2008, 2016, 2019). A possible explanation to the heighten aggression of male mice during the ethanol sensitization procedure is related to social hierarchy. Male mice living in groups tend to establish stable social hierarchies with dominant and subordinate individuals (Arndt et al. 2009; Bartolomucci et al. 2004, 2005; Haemisch et al. 1994). The disinhibiting effects of ethanol could disrupt the established hierarchy (Hilakivi et al. 1989; Hilakivi and Lister 1989; Miczek et al. 1998, 2001) and lead to increased fighting. It is therefore difficult to decide whether housing male Swiss mice in groups is beneficial in terms of stress and welfare and the question is still debated (Kappel et al., 2017). However, it has been repeatedly shown that male mice prefer the company of dominant conspecifics over isolation housing (Van Loo et al. 2001a; Loo et al. 2003), suggesting that social isolation is a stronger chronic stress. In any case, further studies with different methodologies will be required to sort out this question.

In agreement with the above considerations, one might conclude from the present results that groups of eight female mice are an optimal housing condition in female Swiss mice to reduce stress, but not to study the stimulant and sensitized effects of ethanol. For the latter purpose, isolated female mice or mice housed in pairs are better suited to obtain significant levels of sensitized ethanol stimulant effects after a few injections. In most recent published studies on ethanol sensitization, mice were usually housed in groups of 4–5 mice per cage (e.g. Ferreira et al. 2021; O'Brien et al. 2018; Stevenson et al. 2019), which could have reduced the size of the sensitized response. In the sensitization studies from our laboratory, mice are usually housed in pairs (Didone et al. 2008, 2016, 2019; Quoilin et al. 2012b) to compromise with ethical welfare considerations and avoid complete social isolation. Depending on strain, it is often recommended to house mice in groups of two to five per cage for females and three to four for males (Kappel et al., 2017; Olsson & Westlund, 2007). The present results suggest that, at least in female Swiss mice, eight individuals per cage is an adequate housing condition, provided that sufficient cage ground size is granted. In male mice, however, such group housing increases interindividual aggression, especially during an ethanol sensitization procedure.

Based on previous studies (Araujo et al. 2005; Camarini et al. 2018; Kappel et al., 2017; Olsson & Westlund, 2007; Päivärinta 1990; Rueda et al. 2012), it was hypothesized that proper social conditions, i.e. increasing the size of housing groups, would reduce the rate of ethanol sensitization development in mice. Indeed, previous studies showed that environmental enrichment of housing lead to a reduction in drug-seeking, conditioned place preference, relapse and behavioral sensitization to cocaine (Chauvet et al. 2009, 2009; Freese et al. 2018; Lespine and Tirelli 2015; Powell et al. 2020; Solinas et al. 2008, 2009), amphetamines (Browman et al. 1998; Holgate et al. 2017; Sikora et al. 2018; Stairs et al. 2017), nicotine (Ewin et al., 2015; Hamilton et al. 2014; Mesa-Gresa et al. 2013; Redolat et al., 2009) and heroin (Barrera et al. 2021; Galaj et al. 2016). However, such previous studies mainly focused on physical enrichment. To date only two studies tested the effects of social housing conditions on ethanol-induced behavioral sensitization (see Araujo et al. 2005; Päivärinta 1990) and showed that both social isolation and overcrowding potentiate behavioral sensitization to the locomotor activating effect of ethanol. The present results did not confirm such results as all groups of mice developed robust levels of ethanol sensitization at similar rates. What differed between groups were the initial levels of acute ethanol stimulant effects and the achieved levels of stimulant effects after repeated ethanol injections. However, as discussed in Didone et al. (2019), the sensitized levels of ethanol stimulation obtained after a given number of repeated ethanol injections might be as important as the rate at which ethanol sensitization develops. Indeed, most female mice (90%) from vulnerable strains, such as Swiss and DBA mice, developed high levels of ethanol sensitization with enough repeated ethanol injections, but differed in the sensitized levels of ethanol stimulation reached after 8–10 ethanol injections. When understood as homologous to the sensitization of ethanol craving in humans, the magnitude of the sensitized response is an important feature of drug addiction. In the present results, the sensitized stimulant effects achieved after 8 ethanol injections is significantly reduced in mice housed in groups of eight, suggesting that social enrichment exerts a protective effect against ethanol-induced locomotor sensitization.

The present results can be interpreted in line with previous studies reporting significant relationships between stress and drug or alcohol abuse. In humans, it is often reported that alcohol is consumed, and abused by some individuals as a self-medication, to cope with stress and especially social stress (Kalivas and Stewart 1991; Keyes et al. 2011, 2012; Koob et al. 2014; Koob and Volkow 2016; Kosten 2011; Sinha 2008). According to that observation, chronic stress conditions would lead to higher levels of alcohol consumption, in turn increasing the risks of alcohol abuse and addiction. The present results suggest that chronic social stress might also stimulate the neurobiological and cognitive mechanisms involved in the development of drug addiction. Drug-induced behavioral sensitization in rodent studies is used as a model of incentive sensitization, a key explanatory concept in recent drug addiction theories (Berke and Hyman 2000; Nestler 2001; Robinson and Berridge 1993). One strength of the behavioral sensitization experimental paradigm is that drug administration dosage is strictly controlled, thereby allowing interpretations beyond changes in self-administration behaviors. As shown in the present results, higher levels of ethanol sensitization were observed in socially isolated mice relative to mice housed in groups without changes in the quantity or frequency of ethanol self-administration. This suggests that social isolation in humans, as opposed to better social conditions, might contribute to the development of alcohol addiction by direct mechanisms, in addition to changes in alcohol consumption behaviors to cope with stress. Further studies will be required to investigate these mechanisms that might involve stress-alcohol cross-sensitization (Kawakami et al. 2007; Santos-Rocha et al. 2018) and interferences with dopamine neurotransmission (Broadbent et al. 2005; Camarini et al. 2011; Camarini and Pautassi 2016; Didone et al. 2016). In conclusion, the results of the present study show that ethanol acute and sensitized stimulant effects are affected by social housing conditions in mice. Higher ethanol stimulant effects were observed in isolated mice both males and females. These effects were significantly reduced in mice housed in larger groups. Whereas the rate of sensitization per se was not affected by social housing conditions, mice housed in larger groups showed reduced levels of sensitized ethanol stimulant effects at the end of the sensitization procedure. These results agree with previous human studies showing that chronic stress and especially chronic social stress is a risk factor for the development of alcohol abuse and dependence (Keyes et al. 2012; Wojdala et al. 2020).

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Declarations

Competing interests The authors declare no conflict of interest.

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