Basic Science
Research Paper

OPEN

PAIN REPORTS

Inflammatory pain affects alcohol intake in a dose-dependent manner in male rats in the intermittent access model

Yolanda Campos-Jurado[a,b,c], Jose A. Morón[a,b,c,d,e,*]

Abstract

Introduction: Epidemiological studies have shown that there is a relation between pain and alcohol use disorder (AUD). Persistent pain is directly correlated with an increment in alcohol consumption and an increased risk of developing an AUD. Greater levels of pain intensity and unpleasantness are associated with higher levels of relapse, an increase in alcohol consumption, rates of hazardous drinking, and delay to seek for treatment. However, this interaction has not been deeply studied in the preclinical setting.

Methods: Here, we aim to evaluate how inflammatory pain affects levels of alcohol drinking in male and female rats with a history of alcohol. For that, we used an intermittent access 2-bottle choice paradigm combined with the complete Freund Adjuvant (CFA) model of inflammatory pain.

Results: Our results show that CFA-induced inflammatory pain does not alter total intake of 20% alcohol in male or female rats. Interestingly, in males, the presence of CFA-induced inflammatory pain blunts the decrease of alcohol intake when higher concentrations of alcohol are available, whereas it does not have an effect on intake at any concentration in female rats.

Conclusion: Altogether, this study provides relevant data and constitutes an important contribution to the study of pain and AUD and it highlights the necessity to design better behavioral paradigms in animal models that are more translational and reflect current epidemiological findings.

Keywords: Inflammatory pain, Alcohol, Sex differences

1. Introduction

Pain is a global health problem that affects at least 30% of the population in the United States.[22] Epidemiological studies have shown that there is a relation between pain and alcohol use disorder (AUD),[7] and this relation can be bidirectional such that extended and excessive alcohol consumption has been shown to provoke hyperalgesia due to peripheral neuropathy.[24] This alcohol-induced pain condition has been widely studied in both humans and rodents.[8,24] Similarly, pain also seems to have an effect on alcohol drinking. In fact, clinical studies have shown that the presence of persistent pain is directly correlated with an increase in alcohol consumption and an elevated risk of developing an AUD.[32,33] Moreover, in patients with AUD, higher levels of pain have been correlated with higher levels of relapse.[15] Evidence also indicates that greater levels of pain intensity and unpleasantness are associated with an increase in alcohol consumption, rates of hazardous drinking, and delay to seek for treatment.[3,19]

However, despite the epidemiological evidence, the effect that pain has on AUD has not been deeply studied in the preclinical setting. In addition, among the very few studies that have explored this, the results seem to be inconsistent. Some authors have shown that the presence of pain may increase alcohol intake levels in male mice.[4,10,34] By contrast, Lorente et al., reported that only female rats under the pain condition showed a relapse-like behavior when evaluating the effect of pain developed during abstinence, whereas Bilbao et al., showed that male mice relapse after an abstinence period but when the pain condition is induced

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

[a] *Department of Anesthesiology, Washington University in St. Louis, St. Louis, MO, USA,* [b] *Pain Center, Washington University in St. Louis, St. Louis, MO, USA,* [c] *School of Medicine, Washington University in St. Louis, St. Louis, MO, USA, Departments of* [d] *Neuroscience and,* [e] *Psychiatry, Washington University in St. Louis, St. Louis, MO, USA*

**Corresponding author. Address: Washington University School of Medicine, 660 South Euclid, CSRB 5th Floor, St Louis, MO 63110. Tel.: +1(314) 362-0078. E-mail address: jmoron-concepcion@wustl.edu (J.A. Morón).*

PR9 8 (2023) e1082

http://dx.doi.org/10.1097/PR9.0000000000001082

before the animals have any access to alcohol.[2,21] Interestingly, most of these studies examined the potential effect of pain before the alcohol exposure. Based on the clinical data described above, however, pain may be a risk factor to develop AUD or to increase alcohol consumption in subjects that are not naïve to alcohol. Therefore, it is also important to further explore the effect of pain in animals with a history of alcohol exposure.

In addition, both clinical and preclinical studies suggest that the interaction between pain and alcohol use is gender or sex specific.[3,7] There is evidence of sex differences in pain sensitivity and that women are more likely to develop chronic pain.[20,29,31] In the case of alcohol, men have traditionally reported to drink more alcohol than women, although these differences are narrowing during the past years.[11] Moreover, women with AUD show a higher prevalence of medical and psychiatric comorbidities, such as depression and anxiety. Interestingly, female rodents usually exhibit higher levels of alcohol when normalized by body weight.[18,21,27,28] Altogether, these indicate the need to explore potential sex differences when studying the interaction between pain and AUD.

In this study, we aim to evaluate how inflammatory pain affects levels of alcohol drinking in male and female rats with a history of alcohol consumption, using an intermittent access 2-bottle choice paradigm. Our results show that pain does not affect alcohol intake in female rats. However, in males, pain blunts the decrease on alcohol intake when increasing the alcohol concentrations.

2. Materials and methods

2.1. Animals

All procedures were approved by Washington University and the NIH Animal Care and Use Committee in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Sixty adult male and female Long Evans wild type were used (7–8 week and 260–300 g [male rats] and 170–200 g [female rats] at the beginning of the alcohol intermittent access [IA] model). Rats were individually housed on a 12/12-hour dark/light cycle (lights on at 7:00) and acclimated to the animal facility holding rooms for at least 7 days before any manipulation. The temperature for the holding rooms of all animals ranged from 21 to 24°C, whereas the humidity was between 30% and 70%. Food and water were available ad libitum throughout the experimental period.

2.2. Alcohol intermittent access model and pain induction

In this experiment, animals followed the classical alcohol IA model as previously described[6,21] (**Fig. 1A**). In this study, rats had free access to 20% alcohol along with water 3 times a week for 24 hours, followed by 24 or 48 hours of nonaccess to alcohol. Fresh alcohol (ethanol) solution and water were always used. Alcohol bottles were introduced at 10:00 AM every Monday, Wednesday, and Friday and removed 24 hours later at the same time. The bottles were weighed before and after their introduction to measure total fluid intake and alcohol intake and preference for the alcohol bottle. Rats were weighed every day before the introduction of the bottles to calculate alcohol consumption in g/kg/d. Furthermore, to ensure that the alcohol consumption was not influenced by a place preference, the order of the water and alcohol bottles was alternated each time alcohol was introduced.

Once they reached a stable intake level (week 6), rats received 0.15 mL (for males) or 0.12 mL (for females) subcutaneous injection of the complete Freund adjuvant (CFA) or sterile saline in the plantar surface of the hind paw, without altering the intermittent access schedule. Rats then underwent 3 more weeks after the same IA schedule. Finally, during the fourth, fifth, and sixth week after CFA or saline injections, the alcohol concentration was increased up to 30%, 40%, and 50%, respectively.

2.3. Mechanical nociception assessment

To assess baseline nociception (ie, mechanical hyperalgesia) induced by CFA injections, paw withdrawal thresholds (PWTs) were obtained using an electronic Von Frey Anesthesiometer (IITC Life Science, California, USA). Animals were placed in plexiglass chambers on top of a galvanized steel mesh shelf to permit access to the rats' paws from underneath. The anesthesiometer was used to provoke a flexion reflex followed by a flinch response, and the mechanical threshold pressure in grams (PWT) was recorded. Rats were habituated to the test chambers and von Frey procedure for at least 1 hour 1 week before conducting the baseline test. On assessment days (once a week, starting the week before injection), rats were placed in the plexiglass chambers at least 2 hours after removing the alcohol bottles to mitigate potential lasting analgesic effects of alcohol. Rats were acclimated to chambers for 20 minutes before undergoing the procedure. Once acclimated, measurements of mechanical sensitivity were obtained in triplicates for each paw at 5-minute intervals, alternating between the injected and non-injected paw. Paw withdrawal thresholds were determined by averaging all 3 replicates per each testing session.

2.4. Experimental design and statistical analysis

For this experimental design, 60 rats (30 male and 30 female) were used. The experiment was replicated 3 times, including each treatment condition to confirm the reproducibility of the data.

According to the IA protocol, male and female rats were assigned to one of the treatments (saline or CFA) in a counter-balanced fashion based on the baseline alcohol intake (g/kg).

All the results are expressed in mean ± SEM. After assessing the normality of sample data using D'Agostino and Pearson tests and Shapiro–Wilk tests, statistical significance was taken as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ 731, and $^{****}P < 0.0001$, as determined by 2-way analysis of variance (ANOVA) for repeated measures, followed by the Tukey or Bonferroni *post hoc* test for intrasubjects or between-subjects comparisons, respectively, or unpaired *t* test. Statistical analyses were performed using GraphPad Prism 9.1.0. Data collection and analysis were performed blinded to the conditions of the experiments.

3. Results

3.1. Basal alcohol intake is higher in female rats

Alcohol and water drinking behaviors were evaluated on every consumption day for the 5 weeks before saline or CFA injection. Basal levels were considered as the average of the measurements from the past 3 consumption days.

For the total volume of liquid consumed, calculated as the total volume of water and total volume of alcohol, our results show differences between male and female rats. In the individual consumption sessions (**Fig. 1B**), the ANOVA for repeated measurements detected a main effect of sex ($F [4.343, 251.9] = 3.404$, $P = 0.0005$) and of time ($F [4.343, 251.9] = 3.404$, $P = 0.0080$), but there was not a significant interaction between sex and time ($F [14, 812] = 0.7400$). Next, the Bonferroni *post hoc*

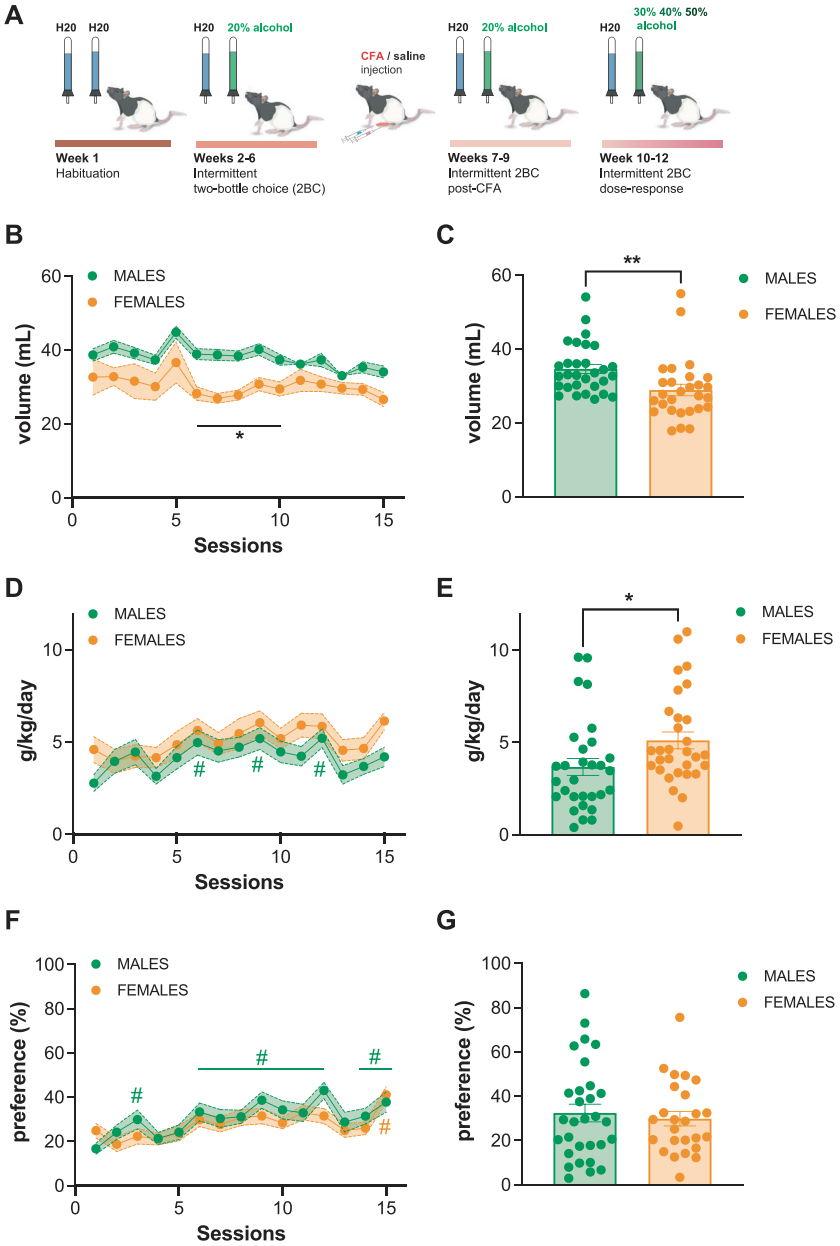

Figure 1. (A) Schematic of the experimental design. (B) Mean ± SEM of total volume of liquid (mL) for the 5 weeks of acquisition for male (green) and female (orange) rats (2-way ANOVA, *$P < 0.05$ Bonferroni *post hoc*). (C) Average mean ± SEM of total volume of liquid (mL) for basal week before CFA/saline injection for male (green) and for female (orange) rats (**$P < 0.01$, *t* test). (D) Mean ± SEM of alcohol intake (g/kg/d) for the 5 weeks of acquisition for male (green) and female (orange) rats (2-way ANOVA, #$P < 0.05$ Tukey's *post hoc*). (E) Average mean ± SEM of alcohol intake (g/kg/d) for basal week before CFA/saline injection for male (green) and for female (orange) rats (*$P < 0.05$, *t* test). (F) Mean ± SEM of alcohol preference (%) for the 5 weeks of acquisition for male (green) and female (orange) rats (2-way ANOVA, #$P < 0.05$ Tukey *post hoc*). (G) Average mean ± SEM of alcohol preference (g%) for basal week before CFA/saline injection for male (green) and for female (orange) rats. ANOVA, analysis of variance; CFA, complete Freund adjuvant.

analysis for multiple comparisons revealed a significant difference of total volume of liquid between males and females in sessions 6 to 10. Moreover, the *t* test showed significant differences in the average of basal week of total volume of liquid between males and females ($P = 0.0056$) (**Fig. 1C**).

When analyzing alcohol intake (calculated as g/kg/d) for the individual consumption sessions (**Fig. 1D**), the ANOVA for repeated measurements detected a main effect of time ($F [14, 812] = 4.393$, $P < 0.0001$) but not of sex ($F [1, 58] = 2.239$, $P = 0.1400$) or a significant interaction of sex and time ($F [14, 812] = 0.9476$, $P = 0.5065$). Interestingly, in the average of basal week, female rats showed significantly higher levels of alcohol intake compared with males (**Fig. 1E**), as it was reported by the *t* test ($P = 0.0304$).

Preference for the alcohol solution was also analyzed for the acquisition period, calculated as the percentage of alcohol volume relative to the total volume consumed. For the individual consumption sessions (**Fig. 1F**), the ANOVA for repeated measurements detected a main effect of time ($F [14, 812] = 10.12$, $P < 0.0001$) and a significant interaction of the sex and time ($F [14, 812] = 1.757$, $P = 0.0409$), but it did not detect differences of the sex ($F [1, 58] = 0.6577$, $P = 0.4207$). When analyzing intrasubject differences, the Tukey *post hoc* analysis for multiple comparisons revealed a significant difference from the first

consumption session in several consumption sessions in males (sessions 3, 6–12, 14 and 15) and in the last consumption session in females (session 15). Finally, no significant differences were found between males and females in preference for the weekly average of basal (**Fig. 1G**), as reported by the *t* test ($P = 0.6293$).

Based on the aforementioned differences on basal week levels of alcohol intake, subsequent male and female data were analyzed separately.

3.2. Inflammatory pain does not alter total intake of 20% alcohol in male or female rats

To explore the effect of inflammatory pain on alcohol intake in our IA procedure, alcohol intake and preference were monitored for 3 weeks after saline or CFA injection and compared with their respective basal levels before the injections (**Fig. 2A**). Moreover, changes in total volume of liquid consumed were also evaluated to ensure that changes in alcohol intake or preference were not due to an alteration in overall animal drinking behavior.

In males, when examining total volume of liquid consumed, the ANOVA for repeated measurements did not detect differences in treatment ($F [1, 28] = 0.9119$, $P = 0.3478$) in the case of individual consumption sessions. However, a main effect of time ($F [6.400, 179.2] = 3.030$, $P = 0.0064$) was detected, as well as a significant interaction of treatment and time ($F [10, 280] = 2.299$, $P = 0.0132$). When comparing with the first basal session, the Tukey *post hoc* analysis for multiple comparisons did not reveal any significant difference in saline or CFA treated males (**Fig. 2B**). When evaluating the weekly average of total volume of liquid consumed in males (**Fig. 2C**), the ANOVA for repeated measures detected a main effect of time ($F [3, 84] = 3.421$, $P = 0.0209$) but did not detect differences in treatment ($F [1, 28] = 0.7878$, $P = 0.3823$) or in the interaction between time and treatment ($F [3, 84] = 1.661$, $P = 0.1817$). When evaluating differences from basal week, the Tukey *post hoc* analysis for multiple comparisons revealed a significant difference in week 3 in saline-treated males ($P = 0.0287$) but not in the CFA group.

Similarly, when comparing the individual sessions of total volume of liquid consumed in females (**Fig. 2D**), the ANOVA for repeated measurements did not detect differences in treatment ($F [1, 28] = 0.9164$, $P = 0.3466$) but a main effect of time ($F [5.939, 166.3] = 3.768$, $P = 0.0016$) and an interaction between time and treatment ($F [10, 280] = 2.483$, $P = 0.0073$) were observed. Subsequent Bonferroni *post hoc* analysis for multiple comparisons revealed a significant difference from the first basal session vs session 1 after CFA injection only in CFA-treated females ($P = 0.0020$) and no intrasubject differences were detected in saline treated females. In the case of the weekly average (**Fig. 2E**), the ANOVA for repeated measures also detected a main effect of time ($F [3, 84] = 4.499$, $P = 0.0056$), but not of treatment ($F [1, 28] = 0.6193$, $P = 0.4379$) or in the interaction between time and treatment ($F [3, 84] = 2.662$, $P = 0.0533$). In addition, the Tukey *post hoc* analysis for multiple comparisons revealed a significant difference from basal week (**Fig. 2A**) in week 2 in saline-treated females ($P = 0.0004$), but no differences from basal week were detected in CFA-treated females.

When assessing overall consumption of alcohol in males (calculated as g/kg/d) for the individual sessions (**Fig. 2F**), the ANOVA for repeated measures detected a main effect of time ($F [6.531, 182.9] = 4.563$, $P = 0.0002$) but did not detect differences in treatment ($F [1, 28] = 0.01580$, $P = 0.9009$) or in the interaction between time and treatment ($F [11, 308] = 1.348$, $P = 0.1972$). When comparing with the first basal session (session −3), the Tukey *post hoc* analysis for multiple comparisons revealed a significant difference from the first basal session (session −3) in session 5 only in CFA-treated males ($P = 0.0456$), and no intrasubject differences were detected in saline-treated males. When analyzing the weekly (**Fig. 2G**), the ANOVA for repeated measures did not detect differences in treatment ($F [1, 28] = 0.01277$, $P = 0.9108$), time ($F [3, 84] = 2.242$, $P = 0.0893$), or in the interaction between treatment and time ($F [3, 84] = 1.070$, $P = 0.3665$).

Similarly, in females and for the levels of alcohol intake in individual sessions (**Fig. 2H**), the ANOVA for repeated measures detected a main effect of time ($F [6.086, 170.4] = 4.661$, $P = 0.0002$) but did not detect differences in treatment ($F [1, 28] = 0.7946$, $P = 0.3803$) or in the interaction between time and treatment ($F [11, 308] = 1.628$, $P = 0.0898$). In this case, the Bonferroni *post hoc* analysis for multiple comparisons revealed a significant difference between saline-treated and CFA-treated females in session 1 after injection ($P = 0.0213$) but did not detect intrasubject differences in either saline-treated or CFA-treated rats. Similar results were found for the weekly average of alcohol intake (**Fig. 2I**). Thus, the ANOVA for repeated measures did not detect differences in treatment ($F [1, 28] = 0.8084$, $P = 0.3763$), time ($F [3, 84] = 1.878$, $P = 0.1396$), or in the interaction between treatment and time ($F [3, 84] = 0.9295$, $P = 0.4302$).

For the preference of the individual consumption sessions in males (**Fig. 2J**), the ANOVA for repeated measures revealed a main effect of time ($F [5.893, 165.0] = 6.022$, $P < 0.0001$), but not of treatment ($F [1, 28] = 0.3554$, $P = 0.5559$), or in the interaction between treatment and time ($F [11, 308] = 0.6955$, $P = 0.7428$). However, when comparing with the first basal session, the Tukey *post hoc* analysis for multiple comparisons did not detect significant differences in saline or CFA-treated males. Next, when assessing the average of preference values weekly (**Fig. 2K**), the ANOVA for repeated measures detected a significant effect of time ($F [3, 84] = 5.916$, $P = 0.0010$), but no differences were detected with respect to treatment ($F [1, 28] = 0.3605$, $P = 0.5531$) or in the interaction between treatment and time ($F [3, 84] = 0.8634$, $P = 0.4634$). Subsequently, Tukey *post hoc* analysis for multiple comparisons revealed significant differences in week 2 when compared with basal week only in saline-treated males ($P = 0.0131$), but not in the CFA-treated group.

For females (**Fig. 2L**), similar results were found for the preference of the individual consumption sessions and the ANOVA for repeated measures did not detect differences in treatment ($F [1, 28] = 0.7665$, $P = 0.3888$) or in the interaction between treatment and time ($F [11, 308] = 1.173$, $P = 0.3053$), but it detected a main effect of time ($F [6.168, 172.7] = 5.670$, $P < 0.0001$). In addition, when comparing the postinjection session with the first basal session, the Tukey *post hoc* analysis for multiple comparisons did not detect significant differences in saline-treated or CFA-treated females. In the case of the average of preference values weekly (**Fig. 2M**), the ANOVA for repeated measures did not detect differences in treatment ($F [1, 28] = 0.7221$, $P = 0.4027$), time ($F [3, 84] = 2.448$, $P = 0.0693$), or in the interaction between treatment and time ($F [3, 84] = 1.718$, $P = 0.1694$).

3.3. Inflammatory pain affects alcohol intake in a dose-dependent manner in male rats

Finally, we explored how inflammatory pain affects the intake when rats are exposed to increasing concentrations of alcohol following the above described IA protocol. To this end, as described in **Figure 3A**, rats underwent 3 additional weeks of our IA procedure in which they were exposed to 30%, 40%, and 50%

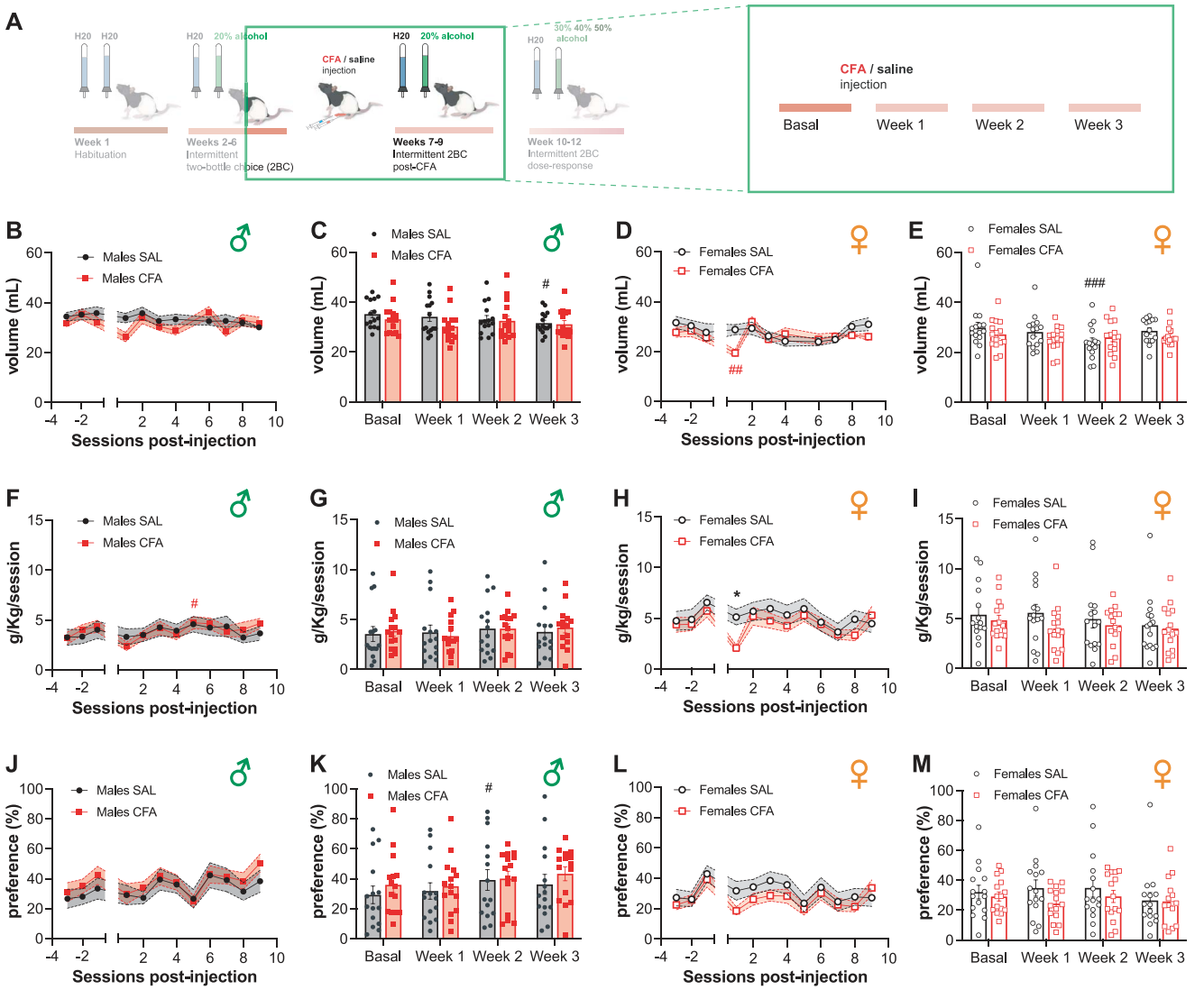

Figure 2. Effect of inflammatory pain on 20% alcohol drinking. Represented are data from basal week and from weeks 1, 2, and 3 after saline (SAL, in black) or CFA (in red) injection for males (full symbols) and females (empty symbols). (A) Schematic of the experimental design. (B) Mean ± SEM of total volume of liquid (mL) for the individual sessions in males. (C) Weekly average mean ± SEM of total volume of liquid (mL) in males (2-way ANOVA, #$P < 0.05$ Tukey *post hoc*). (D) Mean ± SEM of total volume of liquid (mL) for the individual sessions in females (2-way ANOVA, ##$P < 0.01$ Tukey *post hoc*). (E) Weekly average mean ± SEM of total volume of liquid (mL) in females (2-way ANOVA, ###$P < 0.001$ Tukey *post hoc*). (F) Mean ± SEM of alcohol intake (g/kg/d) for the individual sessions in males (2-way ANOVA, #$P < 0.05$ Tukey *post hoc*). (G) Weekly average mean ± SEM of alcohol intake (g/kg/d) in males. (H) Mean ± SEM of alcohol intake (g/kg/d) for the individual sessions in females (2-way ANOVA, *$P < 0.05$ Bonferroni *post hoc*). (I) Weekly average mean ± SEM of alcohol intake (g/kg/d) in females. (J) Mean ± SEM of alcohol preference (%) for the individual sessions in males. (K) Weekly average mean ± SEM of alcohol preference (%) in males (2-way ANOVA, #$P < 0.05$ Tukey *post hoc*). (L) Mean ± SEM of alcohol preference (%) for the individual sessions in females. (M) Weekly average mean ± SEM of alcohol preference (%) in females. ANOVA, analysis of variance; CFA, complete Freund adjuvant.

alcohol, respectively (**Fig. 3A**). Alcohol intake and preference were monitored throughout this time and compared with the levels on the last week of exposure to 20% alcohol (week 3 post-CFA or saline).

We then looked at individual consumption sessions in males for the total volume of liquid during these last weeks of experiments (**Fig. 3B**). The ANOVA for repeated measurements did not detect differences in treatment ($F [1, 28] = 1.238$, $P = 0.2754$), time ($F [2.337, 65.43] = 1.533$, $P = 0.2209$), or on the interaction between time and treatment ($F [11, 308] = 1.333$, $P = 0.2047$). Similarly, for the weekly average of total volume consumed (**Fig. 3C**), the ANOVA for repeated measurements did not detect differences in treatment ($F [1, 28] = 0.5184$, $P = 0.4775$), time ($F [3, 84] = 0.8849$, $P = 0.4524$), or in the interaction between time and treatment ($F [3, 84] = 0.8324$, $P = 0.4798$).

In the individual consumption sessions in females for the total volume of liquid (**Fig. 3D**), the ANOVA for repeated measures revealed a significant interaction for treatment and time ($F [11, 308] = 1.889$, $P = 0.0401$). However, it did not detect differences in treatment factor ($F [1, 28] = 1.664$, $P = 0.2076$) or in time ($F [6.860, 192.1] = 1.243$ $P = 0.2816$). Moreover, the Tukey *post hoc* analysis for multiple comparisons did not detect differences between saline and CFA groups in any of the sessions or intrasubject differences when comparing with the first session when rats were exposed to 20% alcohol. In the case of the weekly average (**Fig. 3E**), no differences were detected by the ANOVA for repeated measures in treatment ($F [1, 28] = 1.664$, $P = 0.2076$), time ($F [3, 84] = 1.077$, $P = 0.3634$), or on the interaction between time and treatment ($F [3, 84] = 0.3495$, $P = 0.7896$).

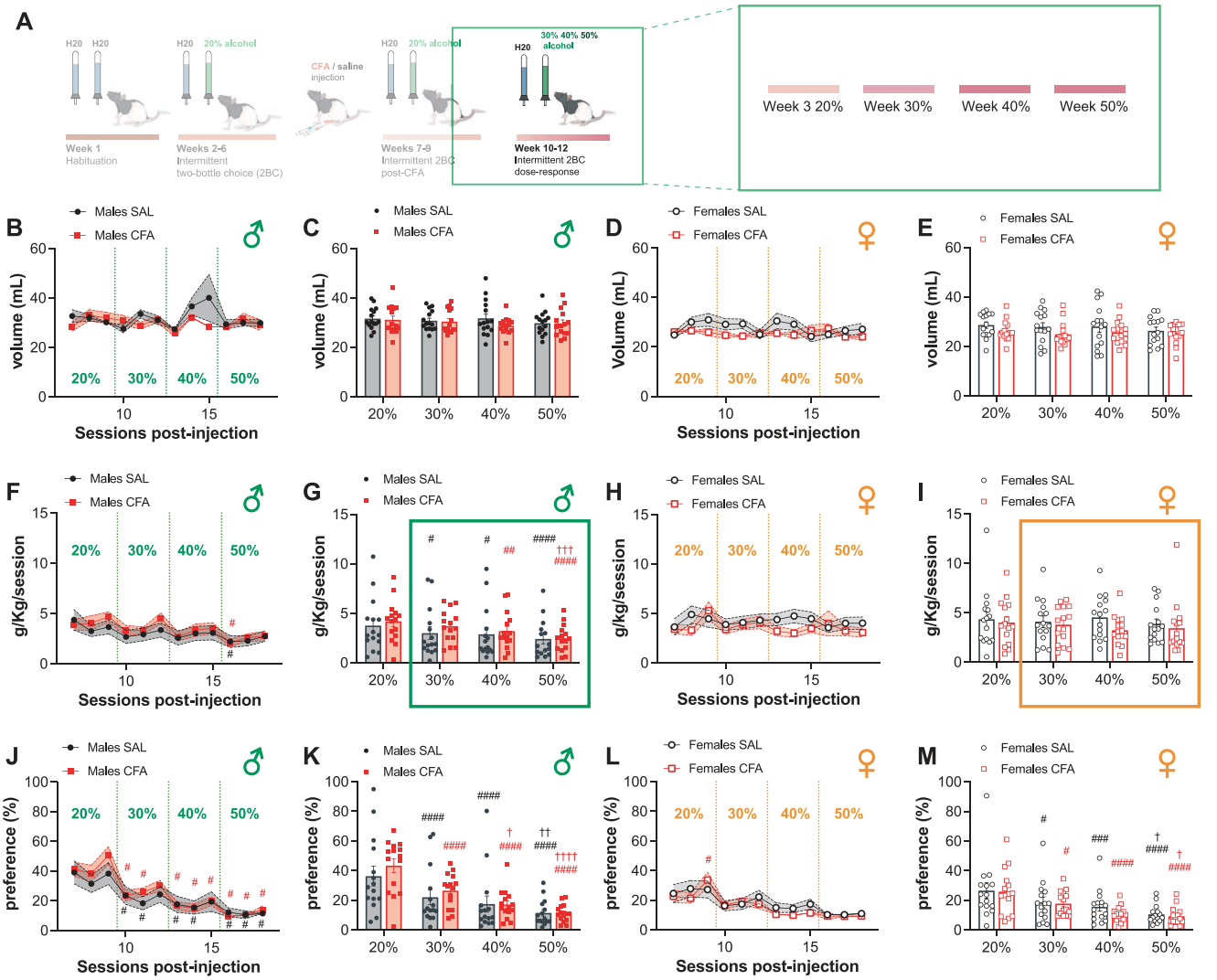

Figure 3. Effect of inflammatory pain on alcohol consumption of different alcohol concentrations. Represented are data from the last week of 20% and from weeks of 30%, 40%, and 50% for saline (SAL, in black) and CFA (in red) groups and for males (full symbols) and females (empty symbols). (A) Schematic of the experimental design. (B) Mean ± SEM of total volume of liquid (mL) for the individual sessions in males. (C) Weekly average mean ± SEM of total volume of liquid (mL) in males. (D) Mean ± SEM of total volume of liquid (mL) for the individual sessions in females. (E) Weekly average mean ± SEM of total volume of liquid (mL) in females. (F) Mean ± SEM of alcohol intake (g/kg/d) for the individual sessions in males (2-way ANOVA, #$P < 0.05$ Tukey *post hoc*). (G) Weekly average mean ± SEM of alcohol intake (g/kg/d) in males (2-way ANOVA, #$P < 0.05$, ##$P < 0.01$, ####$P < 0.0001$ vs 20%, †††$P < 0.001$ vs 50%, Tukey *post hoc*). (H) Mean ± SEM of alcohol intake (g/kg/d) for the individual sessions in females. (I) Weekly average mean ± SEM of alcohol intake (g/kg/d) in females. (J) Mean ± SEM of alcohol preference (%) for the individual sessions in males (2-way ANOVA, #$P < 0.05$ Tukey *post hoc*). (K) Weekly average mean ± SEM of alcohol preference (%) in males (2-way ANOVA, ####$P < 0.0001$ vs 20%,††$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ vs 50%, Tukey *post hoc*). (L) Mean ± SEM of alcohol preference (%) for the individual sessions in females (2-way ANOVA, #$P < 0.05$ Tukey *post hoc*). (M) Weekly average mean ± SEM of alcohol preference (%) in females (2-way ANOVA, #$P < 0.05$, ###$P < 0.001$, ####$P < 0.0001$ vs 20%, †$P < 0.05$ vs 50%, Tukey *post hoc*). ANOVA, analysis of variance; CFA, complete Freund adjuvant.

When analyzing alcohol intake in males for the individual sessions (**Fig. 3F**), the ANOVA for repeated measures revealed a main effect of time (F [6.219, 174.1] = 9.286, $P < 0.0001$), but not of treatment (F [1, 28] = 0.2651, $P = 0.6107$) or in the interaction between treatment and time (F [11, 308] = 1.186, $P = 0.2959$). In addition, when comparing with the first session of the 20% dose week, the Tukey *post hoc* analysis for multiple comparisons revealed a significant difference for the first session of the 50% in both saline ($P = 0.0421$) and CFA groups ($P = 0.0497$). However, when comparing the weekly average of alcohol intake in males (**Fig. 3G**), the ANOVA for repeated measures revealed a main effect of dose (F [3, 84] = 20.20, $P < 0.0001$) but not treatment (F [1, 28] = 0.2645, $P = 0.6111$) or a significant interaction between treatment and dose (F [3, 84] = 0.8702, $P = 0.4600$). Interestingly, the Tukey *post hoc* analysis for multiple comparisons revealed a significant decrease (as compared with 20% alcohol concentration), for the 30% ($P = 0.0404$), 40% ($P = 0.0142$) and 50% ($P < 0.0001$) doses in saline-treated males. However, in CFA-treated males, the *post hoc* only detected differences for the 40% ($P = 0.0041$) and 50% ($P < 0.0001$) doses when compared with 20%, but not for the 30% dose ($P = 0.3263$). Moreover, in CFA-treated males the *post hoc* analysis also detected a significant difference between weeks when rats were exposed to 30% and 50% ($P = 0.0003$) alcohol concentrations.

Surprisingly, different results were found in females. For example, for the alcohol intake in the individual sessions (**Fig. 3H**), the ANOVA for repeated measurements did not detect differences in treatment (F [1, 28] = 0.7631, $P = 0.3898$), time (F [4.369, 122.3] = 1.243, $P = 0.2953$), or in the interaction

between time and treatment (F [11, 308] = 1.279, $P = 0.2357$). In a similar manner, for the weekly data in females (**Fig. 3I**), the ANOVA for repeated measures did not detect differences in treatment (F [1, 28] = 0.7635, $P = 0.3897$), dose (F [3, 84] = 0.6163, $P = 0.6063$), or in the interaction between treatment and dose (F [3, 84] = 0.7540, $P = 0.5231$).

When evaluating the alcohol preference in the individual sessions in male groups (**Fig. 3J**), the ANOVA for repeated measures detected a main effect of time (F [4.999, 140.0] = 34.99, $P < 0.0001$), but not treatment (F [1, 28] = 0.2605, $P = 0.6137$) or a significant interaction between the treatment and time (F [11, 308] = 1.326, $P = 0.2085$). Furthermore, when comparing with the first session when rats were exposed to 20% alcohol concentration, the Tukey *post hoc* analysis for multiple comparisons revealed a significant decrease of the alcohol preference for sessions 10, 11, 13, 14, and 16 to 18 postinjection in the saline group ($P < 0.05$) and for sessions 10, 11, and 13 to 18 in the CFA group ($P < 0.05$). When examining the preference that males exhibited weekly (**Fig. 3K**), the ANOVA for repeated measures detected a main effect of dose (F [3, 84] = 64.08, $P < 0.0001$), but not for treatment (F [1, 28] = 0.2605, $P = 0.6138$) or the interaction between dose and time (F [3, 84] = 1.426, $P = 0.2410$). Moreover, when comparing with the dose of 20% alcohol, the Tukey *post hoc* analysis for multiple comparisons revealed a significant decrease when for the 30%, 40%, and 50% doses in both saline-treated and CFA-treated animals ($P < 0.0001$). When compared with the 50% alcohol dose, the *post hoc* analysis revealed a significant difference with the 30% dose in the saline group ($P = 0.0048$) and for the 30% and 40% doses in the CFA group ($P < 0.05$).

Similarly, for the alcohol preference in the individual sessions in females (**Fig. 3L**), the ANOVA for repeated measures revealed a main effect of time (F [3.576, 100.1] = 16.13, $P < 0.0001$), but not treatment (F [1, 28] = 0.2923, $P = 0.5930$) or a significant interaction between the treatment and time (F [11, 308] = 1.222, $P = 0.2710$). However, when comparing with the first session when rats were exposed to 20% alcohol concentration, the Tukey *post hoc* analysis for multiple comparisons only revealed a significant difference for day 9 postinjection in the CFA group ($P = 0.0401$). Finally, for the weekly average (**Fig. 3M**), the ANOVA for repeated measures did not detect significant differences for treatment (F [1, 28] = 0.2921, $P = 0.5931$) or for the interaction of treatment and dose (F [3, 84] = 0.5527, $P = 0.6477$), but it revealed a main effect of dose (F [3, 84] = 26.44, $P < 0.0001$). When compared with the 20% concentration, the Tukey *post hoc* analysis for multiple comparisons revealed a significant decrease for the 30%, 40%, and 50% ($P < 0.05$) doses and between the 50% and 30% doses ($P < 0.05$) in both saline-treated and CFA-treated females.

3.4. Mechanical nociception hypersensitivity is unaltered in complete Freund adjuvant–injected rats throughout the experimental procedure

The von Frey test showed that mechanical PWTs were lower in both male (**Fig. 4A**) and female (**Fig. 4B**) rats under pain condition until the end of the experimental procedure. The ANOVA for repeated measures detected significant differences in treatment (males: F [1, 28] = 55.07, $P < 0.0001$; females: F [1, 28] = 35.88, $P < 0.0001$) and time (males: F [3.691, 103.4] = 5.459, $P = 0.0007$; females: F [2.575, 72.11] = 13.60, $P < 0.0001$) variables and in the interaction between time and treatment (males: F [5, 140] = 8.917, $P < 0.0001$; females: F [5, 140] = 10.95, $P < 0.0001$). The Bonferroni *post hoc* test confirmed that basal mechanical nociception was not different between groups (males and females: $P > 0.9999$). However, it revealed a significant decrease on days 5 to 35 after injection (males and females: $P < 0.05$) in comparison with the saline-treated rats in both male and female groups. Moreover, when comparing with basal levels, the Tukey *post hoc* analysis for multiple comparisons revealed a significant decrease of the mechanical nociception for days 5 to 35 postinjection only in CFA-treated males and females ($P < 0.05$).

4. Discussion

Pain and AUD are 2 major health problems that can interfere with each other.[3,7,8,15,19,24,32,33] Epidemiological findings suggest that pain may constitute a risk factor for heavy drinking, AUD, and relapse[15,19,32,33] and that can differentially affect men and women.[3,7] However, there is a lack of preclinical research on this topic that could help us understand the specific effects of pain on alcohol-related behaviors and the potential sex differences. Here, we conducted a thorough study in which we assessed the effect of chronic inflammatory pain on alcohol drinking in male and female rats with a history of alcohol exposure. Our results show that pain does not alter alcohol drinking behavior in females. In male rats, however, the presence of inflammatory pain blunts the decrease of alcohol intake when higher concentrations of alcohol are available (**Fig. 5**).

In our paradigm, after 5 weeks of exposure to alcohol following the IA model, inflammatory pain by injection of CFA in the hind paw was induced in half of our animals. Interestingly, during the following 3 weeks that the rats had access to 20% alcohol, we did not detect differences in total weekly alcohol intake or in the weekly preference for the alcohol bottle, when compared with the control (no pain) group. Interestingly, in females, we detected a decrease of alcohol intake only in the first session after CFA injection. However, this difference was not persistent when looking at the average of that week. Based on the schedule of our paradigm, this 1 time point event may not be a representative of the overall effect of pain on alcohol drinking behaviors. To the best of our knowledge, this is the first study in which the induction of pain is produced after the acquisition period using a 2-bottle choice model in rodents. A recent study in mice showed that capsaicin (an acute model of inflammatory pain) did not alter intake of 10% alcohol when mice were exposed to only 1 bottle with alcohol for 2 hours a day.[14] Moreover, Fucich et al.[9] reported that rats in pain generated from traumatic brain injury had a higher breakpoint for 10% alcohol in a progressive ratio session after being trained in a self-administration paradigm. These different results are likely due to differences in species, the model of pain, or the alcohol paradigm. In addition, the previously mentioned studies were only performed in male animals.

The interaction between pain and substance abuse has been deeply explored in the last few decades. Previous data from our laboratory show that rats under inflammatory pain (using the CFA model) self-administer higher amounts of heroin only when a high dose is available, as a consequence of a pain-induced decrease in mu-opioid receptor function in the mesolimbic pathway.[13] There is evidence that the mechanism of action of alcohol is at least partially mediated by mu-opioid receptors (reviewed in Ref. 25). Moreover, alcohol-induced dopamine release is blocked by the presence of inflammatory pain.[5] Therefore, it may be possible that, as observed with opioids, pain may have a dose-dependent effect on alcohol drinking behaviors. To address this, in this study, we assessed whether inflammatory pain could in fact affect alcohol intake when rats were exposed to increasing

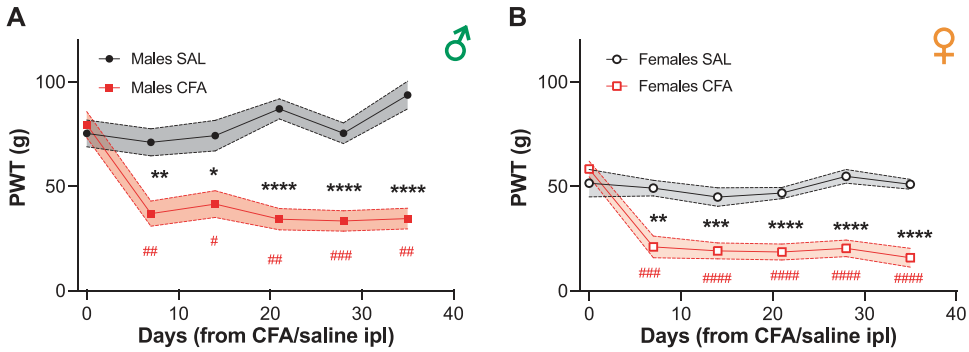

Figure 4. Mean ± SEM of paw withdrawal threshold (PWT) (g) before and after saline (SAL, in black) or CFA (in red) injection for males (full symbols) and females (empty symbols). (A) Mean ± SEM of PAW in males (2-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$, Bonferroni *post hoc*, $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$, Tukey *post hoc*). (B) Mean ± SEM of PAW in females (2-way ANOVA, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, Bonferroni *post hoc*, $\#\#\#P < 0.001$, $\#\#\#\#P < 0.0001$, Tukey *post hoc*). CFA, complete Freund adjuvant.

concentrations such as 30%, 40%, and 50%. Interestingly, we observed that inflammatory pain shifted the dose–response curve in the male group. Saline-treated animals decreased their intake when exposed to increasing alcohol concentrations of 30%, 40%, and 50%, whereas CFA-injected male rats only decreased intake when exposed to concentrations of 40% and 50%. Only one previous study combined similar alcohol concentrations with pain in a long access alcohol model.[26] In this case, chronic neuropathic orofacial pain increased alcohol intake for all doses (10%, 20%, and 40%) in male rats. However, in that study, the pain condition was induced in alcohol naïve rats. That study and this current study show pain effects in a dose-dependent manner in male rats. This seems to be in accordance with previous data showing that inflammatory pain is able to prevent alcohol-induced place preference only at lower doses of alcohol.[5] Together, these data in males indicate that there is a pain-induced upward shift in alcohol dose response, suggesting an increase in hedonic set point, which has been previously shown to constitute an addiction phenotype.[1] Several brain adaptations could be contributing to this shift in the reward valence, including changes in the function of opioid receptors. Previous data have shown that both long exposure to alcohol and pain can dysregulate mu-opioid and kappa-opioid receptors.[13,16,17,23] Therefore, we can hypothesize that the presence of pain could contribute or accelerate these alcohol-induced neuroadaptations in the opioid system.

Our study shows that inflammatory pain did not alter alcohol consumption at any concentration in female rats. These sex differences seem to be in accordance with a recent report showing that pain does not alter the dose response for fentanyl self-administration in female rats.[12] Previous studies have shown differential effects of pain on drug seeking in a sex-dependent manner.[12,30] Therefore, it is likely that similar pain-induced sex-dependent effects may be observed in alcohol drinking behaviors. Moreover, it could be possible that potential sex differences are related to a history of alcohol intake or the time when the pain condition is developed. In this regard, previous studies have shown that when pain is induced in males before any alcohol exposure, they exhibit an increase in alcohol intake after an abstinence period (also called alcohol deprivation effect, ADE).[2]

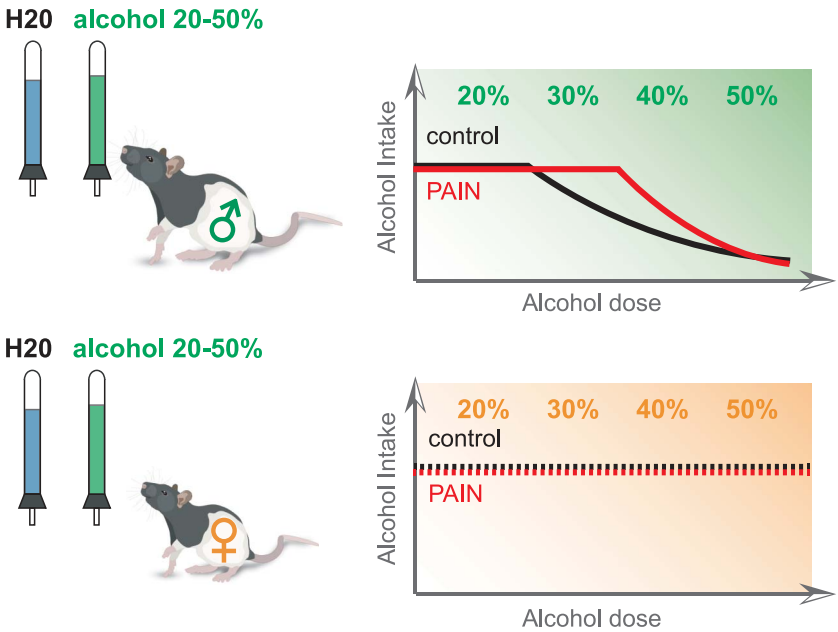

Figure 5. Graphical representation of the obtained results.

Table 1

List of selected preclinical research using rodent models of pain and alcohol drinking.

Species	Strain	Sex	Pain model	Pain onset	Paradigm	Schedule	Session length	Alcohol %	Pain effect	Reference
Mice	C57BL/6J	Both	CFA	After acquisition	2BC	Continuous	24 h	6 → 20%	No control group	30
Mice	C57BL/6 DBA/2	Male	Capsaicin	After acquisition	1B	Daily	2 h	10%	C57BL/6 ↓; DBA/2 ↔	15
Mice	C57BL/6J	Both	CFA	Before	2BC	Continuous	24 h	20%	Males ↑; females ↔	34
Mice	C57BL/6N	Both	SIN	Before	2BC	Continuous	24 h	12%	Acquisition ↔ ADE only males	3
Mice	C57BL/6J	Male	DMM	Before	2BC	Continuous	24 h	2.5 → 20%	↑ for 20%	5
Mice	CD1	Male	PSNL	Before	DID (1B)	Daily	3 h	20%	↑	11
Mice	C57BL/6	Both	mNC	Before	Brief-access	1 test	20 min	1.5% → 40%	↔	2
Rat	Wistar	Male	TBI	After acquisition	Self-admin	FR1	30 min	10%	↑ breakpoint in PR	10
Rat	Long-Evans	Both	CFA	After acquisition	2BC	Intermittent	24 h	20%, 30%, 40%, 50%	20% ↔; DR males resistant ↓	Present
Rat	Wistar	Male	CFA	Before	2BC	Intermittent	24 h	20%	↔	1
Rat	Wistar	Male	CCI	Before	2BC	Intermittent	24 h	10%	↓ and ↑	28
Rat	Wistar	Male	CNOP	Before	DID	Continuous	3 h	10%, 20%, 40%	↑ for all doses	24
Rat	Wistar	Male	CFA	Abstinence	4BC	Continuous	24 h	5%, 10%, 20%	↔ in ADE	6
Rat	Sprague- Dawley	Both	CFA	Abstinence	2BC	Intermittent	24 h	20%	Females ADE; Males ↔	20

↓, decrease; ↑, increase; ↔, no difference; ADE, alcohol deprivation effect; BC, bottle choice; CCI, sciatic nerve constriction; CFA, complete Freund adjuvant; CNOP, chronic neuropathic orofacial pain; DID, drinking in the dark; DMM, destabilization of the medial meniscus; DR, dose response; mNC, constriction of the mental nerve; PR, progressive ratio; PSNL, partial sciatic nerve ligation; SIN, spared nerve injury; TBI, traumatic brain injury.

However, a recent study has shown that when pain is induced during the abstinence period, only female rats exhibit this behavior.[21]

In light of the conflicting previous findings and our own data and with the objective to conduct a thorough review of the existing literature, we performed a comprehensive search of studies that evaluated alcohol drinking behaviors in rodents in the setting of pain. As a result, we found 13 studies, 7 were conducted using mice and 6 were performed in rats. We have summarized the specifics and the outcomes of those studies in **Table 1** and included the results of this study. After summarizing the outcomes from these studies, however, it was challenging to arrive at any significant conclusions as the results seemed to be conflicting and inconsistent. This is likely because of a variety of factors which included the experimental conditions, the pain model, and inclusion of both sexes and the species used. This review of the literature also highlights the fact that the type of pain, its onset, the alcohol drinking paradigm, or even the alcohol concentration used are extremely important when assessing the findings. For example, the highlighted studies used a variety of pain models including inflammatory, neuropathic, or traumatic brain injury–associated pain. In addition, the onset of the pain conditions were not consistent in relation to the timeline of the alcohol exposure with some studies inducing pain in alcohol naïve animals, whereas other studies assessed the effect of pain after acquisition of alcohol drinking or during abstinence. Furthermore, most of the studies used the 2-bottle choice paradigm but with differences in the length of the session which varied from 3 to 24 hours, either in an intermittent or a continuous access. Interestingly, in 3 of the studies, rats were not given the option to choose between an alcohol and a water bottle, and one study used an operant self-administration model. Moreover, the concentrations of alcohol also varied from 1.5% up to 40% as compared with our study that used a maximum concentration of 50%. Finally, only 6 studies including ours incorporated female animals. Thus, the lack of studies including females prevents a thorough examination of potential sex differences in the effects of pain on alcohol drinking behavior.

Altogether, this study provides relevant data and constitutes an important contribution to the study of pain and AUD. Our findings show that inflammatory pain does not affect alcohol drinking behavior in male and female rats with a previous exposure to alcohol. However, when rats are exposed to higher alcohol concentrations, males are more resistant to reduce their total intake when compared with their saline-treated litter mates. Moreover, our review of the existing preclinical data in pain and alcohol drinking behaviors highlights the necessity to better understand the effect of pain on AUD and to design better behavioral paradigms in animal models that are more translational and reflect current epidemiological findings.

Disclosures

The authors have no conflict of interest to declare.

Acknowledgements

The authors thank Dr. Lucía Hipólito for her theoretical contribution to the conceptualization of this project and assistance with training. The authors thank Justin Meyer for breeding the colonies used in these experiments and for general support throughout the experiments. Finally, the authors thank Haziq Latif-Jangda and Aries Ballard for their technical assistance. This work was supported by US National Institutes of Health (NIH) grants DA054900 (JM), DA041781 (JM), DA042581 (JM), DA042499 (JM), DA041883 (JM), DA045463 (JM), and NARSAD Independent Investigator Award from the Brain and Behavior Research Foundation (JM).

Author contributions: Conceptualization: Y. Campos-Jurado and J. A. Morón; Methodology: Y. Campos-Jurado and J. A. Morón; Formal analysis of all data: Y. Campos-Jurado; Alcohol 2 bottle choice and Von Frey: Y. Campos-Jurado; Writing of original draft:

Y. Campos-Jurado and J. A. Morón; Funding acquisition and resources: J. A. Morón; Supervision: Y. Campos-Jurado and J. A. Morón.

Article history:
Received 14 March 2023
Received in revised form 27 April 2023
Accepted 15 May 2023

References

[1] Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. Science 1998;282:298–300.
[2] Bilbao A, Leixner S, Wei S, Cantacorps L, Valverde O, Spanagel R. Reduced sensitivity to ethanol and excessive drinking in a mouse model of neuropathic pain. Addict Biol 2019;24:1008–18.
[3] Boissoneault J, Lewis B, Nixon SJ. Characterizing chronic pain and alcohol use trajectory among treatment-seeking alcoholics. Alcohol 2019;75:47–54.
[4] Butler RK, Knapp DJ, Ulici V, Longobardi L, Loeser RF, Breese GR. A mouse model for chronic pain-induced increase in ethanol consumption. PAIN 2017;158:457–62.
[5] Campos-Jurado Y, Lorente JD, González-Romero JL, Granero L, Polache A, Hipólito L. Impaired alcohol-induced dopamine release in the nucleus accumbens in an inflammatory pain model: behavioral implications in male rats. PAIN 2020;161:2203–11.
[6] Carnicella S, Ron D, Barak S. Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. Alcohol 2014;48:243–52.
[7] Edwards S, Vendruscolo LF, Gilpin NW, Wojnar M, Witkiewitz K. Alcohol and pain: a translational review of preclinical and clinical findings to inform future treatment strategies. Alcohol Clin Exp Res 2020;44:368–83.
[8] Egli M, Koob GF, Edwards S. Alcohol dependence as a chronic pain disorder. Neurosci Biobehav Rev 2012;36:2179–92.
[9] Fucich EA, Mayeux JP, McGinn MA, Gilpin NW, Edwards S, Molina PE. A novel role for the endocannabinoid system in ameliorating motivation for alcohol drinking and negative behavioral affect after traumatic brain injury in rats. J Neurotrauma 2019;36:1847–55.
[10] González-Sepúlveda M, Pozo OJ, Marcos J, Valverde O. Chronic pain causes a persistent anxiety state leading to increased ethanol intake in CD1 mice. J Psychopharmacol 2016;30:188–203.
[11] Grant BF, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Huang B, Jung J, Zhang H, Fan A, Hasin DS. Prevalence of 12-month alcohol use, high-risk drinking, and *DSM-IV* alcohol use disorder in the United States, 2001-2002 to 2012-2013: results from the national epidemiologic survey on alcohol and related conditions. JAMA Psychiatry 2017;74:911.
[12] Higginbotham JA, Abt JG, Tiech RH, Morón JA. Time-dependent enhancement in ventral tegmental area dopamine neuron activity drives pain-facilitated fentanyl intake in males. bioRxiv 2022.08.19.504549; doi: 10.1101/2022.08.19.504549.
[13] Hipólito L, Wilson-Poe A, Campos-Jurado Y, Zhong E, Gonzalez-Romero J, Virag L, Whittington R, Comer SD, Carlton SM, Walker BM, Bruchas MR, Morón JA. Inflammatory pain promotes increased opioid self-administration: role of dysregulated ventral tegmental area μ opioid receptors. J Neurosci 2015;35:12217–31.
[14] Huh SY, Kim S-G, Kim H-K. Capsaicin reduces ethanol consumption in C57BL/6 but not DBA/2 mice. Clin Psychopharmacol Neurosci 2022;20:343–9.
[15] Jakubczyk A, Ilgen MA, Kopera M, Krasowska A, Klimkiewicz A, Bohnert A, Blow FC, Brower KJ, Wojnar M. Reductions in physical pain predict lower risk of relapse following alcohol treatment. Drug Alcohol Depend 2016;158:167–71.
[16] Karkhanis AN, Huggins KN, Rose JH, Jones SR. Switch from excitatory to inhibitory actions of ethanol on dopamine levels after chronic exposure: role of kappa opioid receptors. Neuropharmacology 2016;110:190–7.
[17] Koob GF. Neurobiology of opioid addiction: opponent process, hyperkatifeia, and negative reinforcement. Biol Psychiatry 2020;87:44–53.
[18] Lancaster FE, Spiegel KS. Sex differences in pattern of drinking. Alcohol 1992;9:415–20.
[19] Lawton J, Simpson J. Predictors of alcohol use among people experiencing chronic pain. Psychol Health Med 2009;14:487–501.
[20] Linnstaedt SD, Walker MG, Parker JS, Yeh E, Sons RL, Zimny E, Lewandowski C, Hendry PL, Damiron K, Pearson C, Velilla M-A, O'Neil BJ, Jones J, Swor R, Domeier R, Hammond S, McLean SA. MicroRNA circulating in the early aftermath of motor vehicle collision predict persistent pain development and suggest a role for microRNA in sex-specific pain differences. Mol Pain 2015;11:66.
[21] Lorente JD, Cuitavi J, Campos-Jurado Y, Montón-Molina R, González-Romero JL, Hipólito L. Kappa opioid receptor blockade in the nucleus accumbens shell prevents sex-dependent alcohol deprivation effect induced by inflammatory pain. PAIN 2022;163:e137–47.
[22] Lucas JW, Connor EM, Bose J. Back, lower limb, and upper limb pain among U.S. adults, 2019. NCHS Data Brief 2021;1–8 doi: 10.15620/cdc:107894.
[23] Massaly N, Copits BA, Wilson-Poe AR, Hipólito L, Markovic T, Yoon HJ, Liu S, Walicki MC, Bhatti DL, Sirohi S, Klaas A, Walker BM, Neve R, Cahill CM, Shoghi KI, Gereau RW, McCall JG, Al-Hasani R, Bruchas MR, Morón JA. Pain-induced negative affect is mediated via recruitment of the nucleus accumbens kappa opioid system. Neuron 2019;102:564–73.e6.
[24] Mellion M, Gilchrist JM, De La Monte S. Alcohol-related peripheral neuropathy: nutritional, toxic, or both?: alcoholic polyneuropathy. Muscle Nerve 2011;43:309–16.
[25] Nutt DJ. The role of the opioid system in alcohol dependence. J Psychopharmacol 2014;28:8–22.
[26] Pérez-Martínez IO, Acevedo-Roque CR, Montes-Angeles CD, Martínez M, Miranda F. Mental nerve injury induces novelty seeking behaviour leading to increasing ethanol intake in Wistar rats. Arch Oral Biol 2019;99:66–72.
[27] Perry TW, Sneddon EA, Reichert AN, Schuh KM, Shand NA, Quinn JJ, Radke AK. Sex, but not early life stress, effects on two-bottle choice alcohol drinking behaviors in mice. bioRxiv 2023.01.21.524642; doi: 10.1101/2023.01.21.524642.
[28] Randall PA, Stewart RT, Besheer J. Sex differences in alcohol self-administration and relapse-like behavior in long-evans rats. Pharmacol Biochem Behav 2017;156:1–9.
[29] Rosen S, Ham B, Mogil JS. Sex differences in neuroimmunity and pain: sex differences in neuroimmunity and pain. J Neurosci Res 2017;95:500–8.
[30] Serdarevic M, Striley CW, Cottler LB. Sex differences in prescription opioid use. Curr Opin Psychiatry 2017;30:238–46.
[31] Sorge RE, Totsch SK. Sex differences in pain: sex differences in pain. J Neurosci Res 2017;95:1271–81.
[32] Von Korff M, Crane P, Lane M, Miglioretti DL, Simon G, Saunders K, Stang P, Brandenburg N, Kessler R. Chronic spinal pain and physical–mental comorbidity in the United States: results from the national comorbidity survey replication. PAIN 2005;113:331–9.
[33] Witkiewitz K, McCallion E, Vowles KE, Kirouac M, Frohe T, Maisto SA, Hodgson R, Heather N. Association between physical pain and alcohol treatment outcomes: the mediating role of negative affect. J Consulting Clin Psychol 2015;83:1044–57.
[34] Yu W, Hwa LS, Makhijani VH, Besheer J, Kash TL. Chronic inflammatory pain drives alcohol drinking in a sex-dependent manner for C57BL/6J mice. Alcohol 2019;77:135–45.