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# Chronic inflammatory pain suppresses alcohol intake and accumbal dopamine response

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ABSTRACT

Alcohol use disorders (AUDs) are influenced by factors that initiate, maintain, and/or induce relapse. Chronic pain is both a risk factor for and consequence of AUD, sharing neurological pathways that affect the mesolimbic dopaminergic system. This study examines how inflammatory pain impacts long-term alcohol intake and mesolimbic dopamine transmission in alcohol-naïve rats. Inflammatory pain was induced in eight-week-old Sprague Dawley rats using complete Freund adjuvant (CFA), while controls received saline. Two protocols were followed: one group had continuous access to 20 % ethanol for one month (n = 10/sex), and the second group for three months (n = 8/sex) in a two-bottle choice paradigm. Mechanical nociception was assessed weekly using the Von Frey test. Dopamine levels in the nucleus accumbens core were measured through microdialysis during the final 1.5 months of ethanol exposure in the second cohort. Due to experimental limitations animals underwent microdialysis at different time points after alcohol was firstly introduced, this was done in a balanced manner by alternating sex and group. After a month of alcohol exposure, rats showed no differences in alcohol consumption. However, from the second month until the end, rats exhibited a non-sex-dependent decrease in alcohol intake, significantly lower in CFA-animals. This reduction was accompanied by a blunted ethanol-evoked dopamine release in the nucleus accumbens. Moreover, low mechanical nociception was maintained until the end of the experiment in CFA-animal. These findings provide insights into the effect of pain on alcohol-elicited neurochemical responses and drinking behaviour, showing how pain alters dopamine response to alcohol, affecting drinking patterns and prolonging nociception from CFA.

## 1. Introduction

Clinical studies have revealed a connection between pain and alcohol use disorders (AUDs), likely due to pain-induced alterations in the function of overlapping brain regions (Apkarian et al., 2013; Jakubczyk et al., 2016). Various types of pain conditions alter the structure and anatomy of the basal ganglia and connected areas which negatively impacts motivation and reward processing, which can trigger stress and negative affect states (Schwartz et al., 2014; Markovic et al., 2021). The Ventral Tegmental Area–Nucleus Accumbens (VTA–NAc) connection, a pathway with numerous dopaminergic neurons, plays a pivotal role in AUDs (Deehan et al., 2013). Of note, the NAc is central to reward, driving motivation and avoiding aversive behaviours (Floresco, 2015). It is divided into the NAc Shell (NAcS) and NAc Core (NAcC), based on innervation and cell types. Dopamine (DA) input from the VTA activates neurons that filter reinforcer-related information. NAcS, part of the extended AMY, processes the "liking" aspect of reward (Zahm, 1999; Castro and Berridge, 2014), while NAcC handles reward-related motor functions, promoting behaviours that lead to further reward exposure (Zahm, 1999). As reviewed by Dahchour and Ward (2022), many researchers have shown that ethanol alters DA extracellular levels within the NAc. They highlight how ethanol-induced DA levels fluctuate during different phases of alcohol exposure, which is relevant to understanding the neurobiological basis of alcohol-related behaviours. Overall, acute ethanol exposure increases DA release (Yoshimoto et al., 2000; Bassareo et al., 2017), chronic exposure induces minimal changes (Weiss et al., 1996; Siciliano et al., 2016), and DA release decreases during withdrawal (Diana et al., 1993; Bailey et al., 2001; Shen, 2003; Zhao et al., 2015). Interestingly, pain inhibits DA transmission in the mesocorticolimbic system (MCLS) (Serafini et al., 2020), in a manner similar

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to the effects of chronic alcohol exposure or withdrawal. Indeed, we previously demonstrated that inflammatory pain is a risk factor for alcohol relapse after a forced withdrawal period (Lorente et al., 2022). Additionally, previous data has shown that inflammatory pain also affects the acquisition (Yu et al., 2019) and maintenance of alcohol intake (Campos-Jurado and Morón, 2023). These studies revealed a strong sex-dependent factor. Notably, males in pain were more prone to have high initial alcohol intake rates and maintain alcohol consumption at high ethanol doses, while females in pain were at greater risk of relapse. However, none of these studies did not explore DA transmission under these conditions. Therefore, in this study, we assess DA release within the NAc in animals with a history of alcohol exposure and observe how long-term inflammatory pain modulates DA transmission.

## 2. Methods

### 2.1. Animal model

8-week-old Sprague Dawley rats (28 animals/sex) were used (Envigo®, Barcelona, Spain). Animals were kept individually housed in standard plastic cages (42 × 27 × 18 $cm^3$) in light/dark (12/12 h) controlled cycles, temperature 23 ± 1 °C, and 60 % humidity with food and tap water provided *ad libitum*. Half of the animals were injected with 0.1 mL of complete Freund's adjuvant (CFA) to induce inflammatory pain, while the other half received saline as a control, with the injected paw alternating between the right and left. Mechanical nociception was monitored by the Von Frey test as previously described (Lorente et al., 2022). Briefly, the protocol began with a 20-min habituation to the behavioural boxes and testing room, followed by the manual application of five filaments (Aesthesio, San José, CA) using a simplified up–down method. The first session was performed just before the intraplantar injection to obtain the basal mechanical nociception. Afterwards, weekly Von Frey sessions were carried out. Protocols were approved by the Animal Care Committee of University of Valencia (2018/VSC/PEA0177) following EEC Council Directive (63/2010) and Spanish law (RD 53/2013).

### 2.2. Experimental design

Two experiments were run. Animals in the Experiment 1 (n = 20/sex) followed the ethanol continuous access for a month, whereas rats included in the Experiment 2 (n = 8/sex) followed the protocol for 3 months. In both experiments animals were given continuous access to tap water and to a 20 % ethanol solution in their home cages. While 20 % ethanol may be aversive, studies show it leads to higher consumption (kg/g/day) than lower concentrations. Notably, Sprague Dawley rats do not reduce intake at this concentration (Martinetti et al., 2006). Moreover, 20 % ethanol has traditionally been used as a model of consumption of spirits, as shown in previous studies from our group (Cuitavi et al., 2021, 2024; Lorente et al., 2022). Bottles were daily weighed to calculate ethanol consumption (g/kg/day), and its position was changed to avoid location preferences. Animals were culled by isoflurane overdose 24 h after the last ethanol exposure.

To further investigate the underlying mechanisms of the CFA effect on ethanol-induced DA release in the NAc in rats with a long-term exposure to alcohol, microdialysis experiments were carried out in the Experiment 2 along the last one and a half month of alcohol consumption (Ethanol bottles were continuously available, except during surgery and microdialysis procedures). Of note, although animals underwent microdialysis at different time points after alcohol was firstly introduced, this was done in a balanced manner. The time when the microdialysis was performed did not elicit distinct effects (Supplementary Figs. 2A and 2B). Moreover, we made sure that animals did not change alcohol intake before and after the microdialysis procedure (Supplementary Fig. 2C). Animals were alternated while ensuring consideration of their sex and whether they had been injected with saline or CFA. Probes were bilaterally implanted into the NAcC (Males - anteroposterior: 1.4 mm, mediolateral: 1.5 mm and dorsoventral: 7.8 mm from bregma; Females - anteroposterior: 1.2 mm, mediolateral: 1.4 mm and dorsoventral: 7.6 mm from bregma; as previously described (Campos-Jurado et al., 2020). Although 16 rats were bilaterally implanted with probes in this brain region, in some cases, probes may not have been correctly positioned, could have become blocked between the surgery and microdialysis days, or may have encountered issues during the experiment. Probes affected by any of these problems were excluded from all analyses. Therefore, only completed experiments were used (n = 10 saline experiments and 8 CFA-experiments). After a stabilisation period of 100 min, samples were collected every 20 min and extracellular DA levels were determined by using offline high performance liquid chromatography (HPLC) with electrochemical detection (Campos-Jurado et al., 2020). Once DA baseline level was established (defined as 3 last consecutive samples presenting a maximum 10 % variation in DA content), 2 mL of vehicle (saline) were subcutaneously (s.c.) administered and DA levels were analysed every 20 min for 100 min as a control for the possible effect of the s.c. injection itself. Right after, a single s.c. ethanol dose (1.5 g/kg diluted in 2 mL of saline) was administered and DA levels in the dialysates were analysed for 160 min more. We selected the highest s.c. dose that did not cause necrosis or irritation at the injection site (Strickley RG, 2004). After animal euthanasia, brains were removed and snap froze in dry ice. Then, we obtained brain 40 μm-thick coronal slices by using a cryostat, which were stained with a cresyl violet protocol to verify probe placement. Paw swelling was assessed post-procedure by measuring and doing a ration of the width (sole to dorsum) of both injected and non-injected paws using a vernier calliper.

### 2.3. Statistical analysis

Results are shown as mean ± standard error of the mean. To perform the statistical analysis, the 26.0 version of the SPSS program was used. The Shapiro-Wilk and Levene tests were performed to assess the normality and the homogeneity of the variance of the data. For the Von Frey test, alcohol intake, DA levels and AUC of DA levels we used a three-way ANOVA since there are three factors defining our groups namely the treatment with either saline or CFA, the sex of the animal, and the time when each piece of data was collected (repeated measures). However, since no sex difference were found for any analysis, we further performed a two-way ANOVA where namely the treatment with either saline or CFA, and the time (repeated measures) were the factors to take into account. The Bonferroni multiple comparisons *post-hoc* test was used when appropriate in all the stated measurements but for the DA levels. In this case, the Dunnett *post-hoc* test was applied. A T-test for independent samples was used to analyse the differences in the widths of the paws. In all cases a 95 % confidence level was set.

## 3. Results

All the statistical analysis has been included in a supplementary table. Since there was no sex differences detected for any experiment (As shown in the graphs in Supplementary Fig. 1), males and females were pooled when graphing.

### 3.1. Experiment 1: effect of CFA on a one-month alcohol intake

The two-bottle choice procedure was used to evaluate the effect of pain on acquisition of alcohol intake in male and female rats. The alcohol drinking behaviour during the acquisition was evaluated on every consumption day (Fig. 1A). The ANOVA did not detect differences between groups along time. Moreover, as expected, mechanical nociceptive thresholds were lower in CFA-treated rats than in saline-treated animals until the end of the experimental procedure (Fig. 1B). The ANOVA detected a main effect on the interaction between time and the

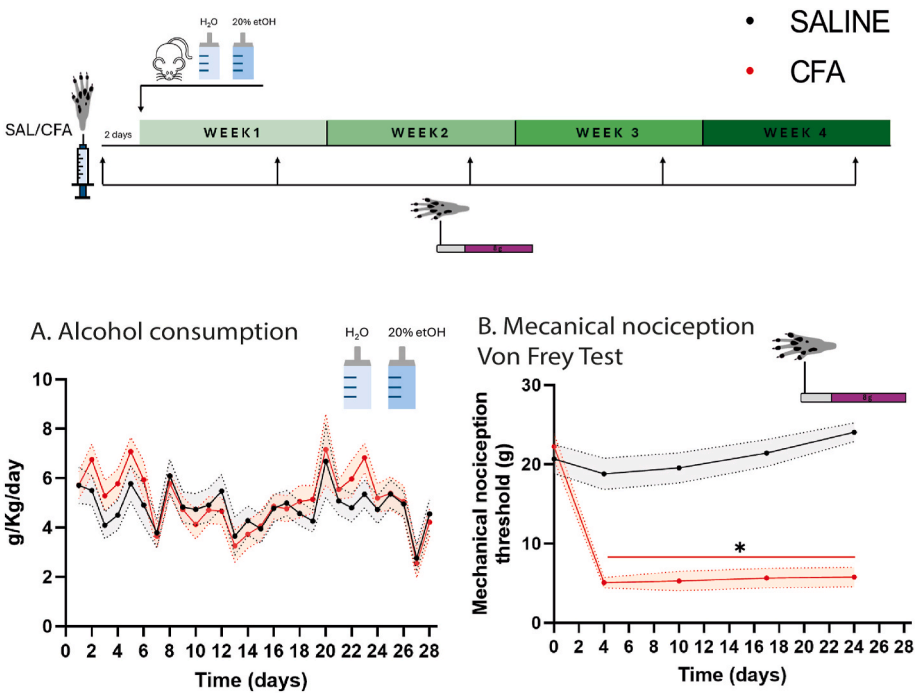


**Fig. 1.** Inflammatory pain modulation of 1-month alcohol consumption patterns (n of animals = 10/sex/pain condition). A) Daily representation of alcohol intake levels (g/kg/day) for 30 days. Data are expressed as mean ± SEM of each consumption day (n = 20/group). B) Mechanical nociception threshold. Data are expressed as mean ± SEM of the mechanical nociception threshold (g) in each Von Frey session (n = 10/group). ANOVA for repeated measures followed by Bonferroni multiple comparisons, *p < 0.05 (Differences with baseline). SAL, saline, CFA, complete Freund's adjuvant, etOH, ethanol.

pain condition. The Bonferroni *post hoc* analysis for multiple comparisons revealed a significant difference between saline and CFA treated animals in days 3, 10, 17 and 24, and significant differences from baseline in all post-CFA measurements.

### 3.2. Experiment 2: effect of CFA on three-month alcohol intake and in the ethanol-induced DA release in the NAc

This group of rats were continuously exposed to ethanol for three months from two days after a s.c. saline or a CFA injection in the plantar surface of the hind paw. Sex did not elicit any significant differences in ethanol intake. Therefore, Fig. 2A shows the average of the daily alcohol intake rate (g/kg/day, n = 8/group) for the saline and the CFA groups in each month of the procedure. The ANOVA indicated statistically significant differences along time and in between saline- and CFA-animals. Interestingly, the *post-hoc* test showed a statistically significant decrease of the alcohol intake levels during the second and the third months when compared to their first month of alcohol consumption. Furthermore, during the third month CFA-animals showed statistically significant lower alcohol consumption levels than saline-animals.

We further investigate DA signalling alterations in NAcC in a sample from these rats exposed to three-months alcohol intake. Our data showed that inflammatory pain blunts ethanol induced NAcC DA release after long-term alcohol exposure (Fig. 2C). The ANOVA revealed that there are statistically significant differences between saline- and CFA-animals. The *post-hoc* test showed a statistically significant increase of DA levels at various times after the s.c. ethanol injection when compared to basal levels only in saline-animals. In fact, results from Fig. 2D, which shows a comparison of AUCs from the DA levels after the s.c. vehicle injection (0–120 min) and the s.c. ethanol injection (120–240 min), confirm this effect. The ANOVA showed statistically significant differences in interaction between a pain condition and time. However, the *post-hoc* test revealed that only in saline-animals a significantly increased the AUC of DA levels after the ethanol injection compared to the AUC of DA levels after the saline injection was observed.

Very interestingly, low mechanical nociception was sustained throughout the study, coinciding with the period in which the animals were drinking (Fig. 2E). In fact, the ANOVA show statistically significant differences in the interaction between the existence of a pain condition and time and the *post-hoc* analysis revealed that, every week after the plantar injection, CFA-animals had a statistically significant lower nociception threshold when compared to their basal levels and saline-animals the same week. Moreover, in these conditions, CFA-animals preserved the inflammation until the end of the experimental procedure (Fig. 2F).

## 4. Discussion and conclusions

The effect of pain on alcohol drinking behaviour in rodents remains controversial and is critically influenced by several factors, including strain, alcohol exposure protocols, and the phases of AUD modelling. Our results show how the temporal evolution of inflammatory pain, combined with the acquisition of alcohol intake, is essential for understanding the long-term changes observed in alcohol consumption. Differences between pain and no-pain rats only become apparent after the first month of the protocol. Indeed, long-term inflammatory pain reduces voluntary alcohol consumption at a 20 % ethanol concentration after three months of a free continuous access paradigm, in both male and female rats. However, evidence shows that C57BL/6J mice experienced an increase in alcohol intake following the same CFA inflammatory pain model and the same continuous access to ethanol as in our study, although they only measured alcohol intake over three weeks (Yu et al., 2019). Notably, mice and rats exhibit different drinking behaviour patterns depending on the alcohol paradigm used (Hopf and Lesscher, 2014) and process pain differently (Mogil, 2019). Therefore, more comparative studies are warranted to better understand how inflammatory pain modulates alcohol drinking behaviours in each rodent species and during different phases of AUD modelling.

Interestingly, and supporting our findings on alcohol drinking, inflammatory pain blunted acute ethanol-evoked DA release in the NAcC

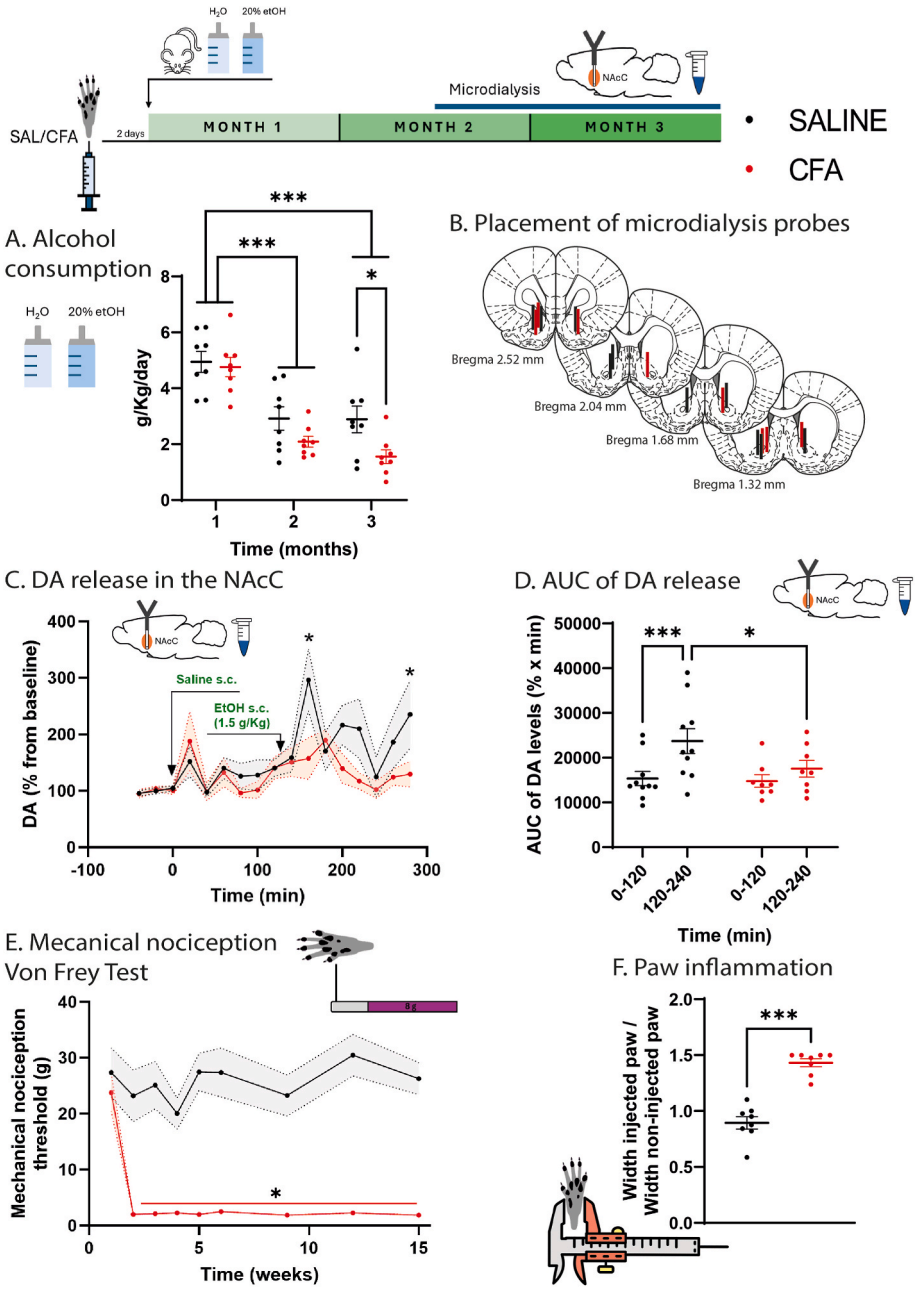


**Fig. 2.** Impact of inflammatory pain on long-term alcohol consumption patterns and ethanol-evoked DA release in the NAcC (n of animals = 4/sex/pain condition). A) Average mean ± SEM of total alcohol intake (g/kg/day) of each week during the alcohol consumption period (n = 8/group). Points represent the individual data from each animal within each group. ANOVA for repeated measures followed by Bonferroni multiple comparisons, *p < 0.05, ***p < 0.005. B) Diagram of coronal sections indicating the placement of microdialysis probes in the NAcC. C) Ethanol-evoked DA release in the NAcC. Data are expressed as mean ± SEM of the percentage of DA from baseline (n = 8–9/group). ANOVA for repeated measures followed by Dunnett, *p < 0.05 (Differences with baseline). D) Global change in DA levels induced by saline (0–120 min) and ethanol (120–240 min) calculated as AUC. Data are expressed as mean ± SEM of the AUC from the percentage of DA from baseline (n = 8–9/group). ANOVA for repeated measures followed by Bonferroni multiple comparisons, *p < 0.05, ***p < 0.005. E) Mechanical nociception threshold. Data are expressed as mean ± SEM of the mechanical nociception threshold (g) in each Von Frey session (n = 8/group). ANOVA for repeated measures followed by Bonferroni multiple comparisons, *p < 0.05 (Differences with baseline). F) Ratio of injected paw width/non-injected paw width. Data are expressed as mean ± SEM of that ratio (n = 8/group). Points represent the individual data from each animal within each group. T-test, ***p < 0.005. DA, dopamine; NAcC, nucleus accumbens core; AUC, area under the curve; SAL, saline, CFA, complete Freund's adjuvant.

of these long-term alcohol-exposed rats, suggesting alterations in alcohol reward processing. Although long-term alcohol exposure has traditionally been associated with low DA levels in the MCLS (Dahchour and Ward, 2022), a recent study using fast-scan cyclic voltammetry showed that high alcohol-drinking mice still increase their DA levels in the NAcC following stimulation (Liu et al., 2020), similar to what we observed in our control rats. Thus, these alterations in DA release after long-term alcohol exposure under pain conditions may underlie the reduction in consumption of the alcohol solution used in the study. This decrease in DA is also compatible with depressive phenotypes associated with chronic pain conditions (Schwartz et al., 2014; Markovic et al., 2021). Moreover, Campos-Jurado et al. (2020) showed that CFA-injected male rats exhibited altered DA release after a single dose of ethanol under acute conditions, correlating with dose-dependent

alterations in ethanol-conditioned place preference. In this regard, pain-induced alterations in the ability of VTA–NAc DA neurons to respond to reward stimuli (Markovic et al., 2021) might regulate alcohol consumption in a dose-dependent manner. Of note, inflammatory pain shifts the dose-response curve to the left for various substances, including alcohol and opioids (Hipólito et al., 2015; Campos-Jurado and Morón, 2023). This suggests that individuals using drugs of abuse may seek higher doses over time. In fact, in rats with a history of alcohol consumption prior to CFA injection, pain did not alter alcohol consumption patterns when 20 % ethanol was used, although it impaired the reduction in alcohol consumption when the animals were challenged with 50 % ethanol (Campos-Jurado and Morón, 2023).

Finally, we present novel evidence that the inflammatory pain model based on a single CFA injection, concomitant with alcohol exposure, lasts for longer periods than previously reported in rats without alcohol exposure (Narita et al., 2006; Parent et al., 2012; Coderre and Laferrière, 2020; Kremer et al., 2021). The CFA model is typically used for a maximum of three weeks, as CFA elicits prompt inflammation, and this duration is sufficient to study chronic pain conditions (Coderre and Laferrière, 2020). Indeed, no previous study has reported using the CFA model for longer than one month. We consider this finding highly significant in the context of AUD and aim to explore it further in future long-term studies.

Our findings demonstrate that inflammatory pain modulates alcohol consumption and its neurochemical effects over time. Specifically, prolonged exposure to alcohol leads to a reduction in intake after the first month in the presence of inflammatory pain. Additionally, CFA-induced inflammation results in sustained enhanced nociception for at least three months during alcohol exposure. Notably, inflammatory pain attenuates ethanol-induced dopamine release in the mesolimbic pathway following one month of ethanol exposure. These results highlight the complex relationship between chronic pain, alcohol consumption, and dopaminergic signalling, with potential implications for understanding substance use in pain conditions.

## CRediT authorship contribution statement

**Javier Cuitavi:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ana Riera-Calabuig:** Investigation, Formal analysis. **Yolanda Campos-Jurado:** Methodology, Investigation, Formal analysis. **Jesús D. Lorente:** Methodology, Investigation, Formal analysis. **María de Jorge:** Investigation. **Ana Polache:** Writing – review & editing, Supervision. **Lucía Hipólito:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

## Ethics approval

Protocols were approved by the Animal Care Committee of University of Valencia following EEC Council Directive (63/2010) and Spanish law (RD 53/2013).

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## Declaration of competing interest

The authors have no conflicts of interest to declare.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2025.105974.

## Data availability

Data will be made available on request.

## References

Apkarian, A.V., Neugebauer, V., Koob, G., Edwards, S., Levine, J.D., Ferrari, L., et al., 2013. Neural mechanisms of pain and alcohol dependence. Pharmacol. Biochem. Behav. 112, 34–41. https://doi.org/10.1016/j.pbb.2013.09.008.

Bailey, C.P., O’Callaghan, M.J., Croft, A.P., Manley, S.J., Little, H.J., 2001. Alterations in mesolimbic dopamine function during the abstinence period following chronic ethanol consumption. Neuropharmacology 41 (8), 989–999. https://doi.org/10.1016/s0028-3908(01)00146-0.

Bassareo, V., Cucca, F., Frau, R., Di Chiara, G., 2017. Changes in dopamine transmission in the nucleus accumbens shell and core during ethanol and sucrose self-administration. Front. Behav. Neurosci. 11, 71. https://doi.org/10.3389/fnbeh.2017.00071.

Campos-Jurado, Y., Morón, J.A., 2023. Inflammatory pain affects alcohol intake in a dose-dependent manner in male rats in the intermittent access model. Pain Rep. 8 (4), e1082. https://doi.org/10.1097/PR9.0000000000001082.

Campos-Jurado, Y., Lorente, J.D., González-Romero, J.L., Granero, L., Polache, A., Hipólito, L., 2020. Impaired alcohol-induced dopamine release in the nucleus accumbens in an inflammatory pain model: behavioral implications in male rats. Pain 161 (9), 2203–2211. https://doi.org/10.1097/j.pain.0000000000001915.

Castro, D.C., Berridge, K.C., 2014. Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness “liking” and “wanting”. J. Neurosci. 34 (12), 4239–4250. https://doi.org/10.1523/JNEUROSCI.4458-13.2014.

Coderre, T.J., Laferrière, A., 2020. The emergence of animal models of chronic pain and logistical and methodological issues concerning their use. J. Neural Transm. 127, 393–406. https://doi.org/10.1007/s00702-019-02103-y.

Cuitavi, J., Campos-Jurado, Y., Lorente, J.D., Andrés-Herrera, P., Ferrís-Vilar, V., Polache, A., Hipólito, L., 2024. Age-and sex-driven alterations in alcohol consumption patterns: role of brain ethanol metabolism and the opioidergic system in the nucleus accumbens. Pharmacol. Biochem. Behav. 244, 173845. https://doi.org/10.1016/j.pbb.2024.173845.

Cuitavi, J., Lorente, J.D., Campos-Jurado, Y., Polache, A., Hipólito, L., 2021. Neuroimmune and mu-opioid receptor alterations in the mesocorticolimbic system in a sex-dependent inflammatory pain-induced alcohol relapse-like rat model. Front. Immunol. 12, 689453. https://doi.org/10.3389/fimmu.2021.689453.

Dahchour, A., Ward, R.J., 2022. Changes in brain dopamine extracellular concentration after ethanol administration; rat microdialysis studies. Alcohol Alcohol 57 (2), 165–175. https://doi.org/10.1093/alcalc/agab072.

Deehan Jr., G.A., Hauser, S.R., Wilden, J.A., Truitt, W.A., Rodd, Z.A., 2013. Elucidating the biological basis for the reinforcing actions of alcohol in the mesolimbic dopamine system: the role of active metabolites of alcohol. Front. Behav. Neurosci. 7, 104. https://doi.org/10.3389/fnbeh.2013.00104.

Diana, M., Pistis, M., Carboni, S., Gessa, G.L., RoSSETTI, Z.L., 1993. Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. Proc. Natl. Acad. Sci. USA 90 (17), 7966–7969. https://doi.org/10.1073/pnas.90.17.7966.

Floresco, S.B., 2015. The nucleus accumbens: an interface between cognition, emotion, and action. Annu. Rev. Psychol. 66 (1), 25–52. https://doi.org/10.1146/annurev-psych-010213-115159.

Hipólito, L., Wilson-Poe, A., Campos-Jurado, Y., Zhong, E., Gonzalez-Romero, J., Virag, L., et al., 2015. Inflammatory pain promotes increased opioid self-administration: role of dysregulated ventral tegmental area μ opioid receptors. J. Neurosci. 35 (35), 12217–12231. https://doi.org/10.1523/JNEUROSCI.1053-15.2015.

Hopf, F.W., Lesscher, H.M., 2014. Rodent models for compulsive alcohol intake. Alcohol 48 (3), 253–264. https://doi.org/10.1016/j.alcohol.2014.03.001.

Jakubczyk, A., Ilgen, M.A., Kopera, M., Krasowska, A., Klimkiewicz, A., Bohnert, A., et al., 2016. Reductions in physical pain predict lower risk of relapse following alcohol treatment. Drug Alcohol Depend. 158, 167–171. https://doi.org/10.1016/j.drugalcdep.2015.11.020.

Kremer, M., Becker, L.J., Barrot, M., Yalcin, I., 2021. How to study anxiety and depression in rodent models of chronic pain? Eur. J. Neurosci. 53 (1), 236–270. https://doi.org/10.1111/ejn.14686.

Liu, Y., Montgomery, S.E., Juarez, B., Morel, C., Zhang, S., Kong, Y., et al., 2020. Different adaptations of dopamine release in nucleus accumbens shell and core of individual alcohol drinking groups of mice. Neuropharmacology 175, 108176. https://doi.org/10.1016/j.neuropharm.2020.108176.

Lorente, J.D., Cuitavi, J., Campos-Jurado, Y., Montón-Molina, R., González-Romero, J.L., Hipólito, L., 2022. Kappa opioid receptor blockade in the nucleus accumbens shell prevents sex-dependent alcohol deprivation effect induced by inflammatory pain. Pain 163 (1), e137–e147. https://doi.org/10.1097/j.pain.0000000000002332.

Markovic, T., Pedersen, C.E., Massaly, N., Vachez, Y.M., Ruyle, B., Murphy, C.A., et al., 2021. Pain induces adaptations in ventral tegmental area dopamine neurons to drive anhedonia-like behavior. Nat. Neurosci. 24 (11), 1601–1613. https://doi.org/10.1038/s41593-021-00924-3.

Martinetti, M.P., Lowery, E.G., Vona, S.R., Wichnick, A.M., Adler, R.A., Finch, D.G., 2006. Limited-access consumption of ascending ethanol concentrations in alcohol-preferring, nonpreferring, and sprague–dawley rats. Alcohol Clin. Exp. Res. 30 (5), 836–843. https://doi.org/10.1111/j.1530-0277.2006.00098.x.

Mogil, J.S., 2019. The translatability of pain across species. Philosoph. Trans. Royal Soc. B 374 (1785), 20190286. https://doi.org/10.1098/rstb.2019.0286.

Narita, M., Kaneko, C., Miyoshi, K., Nagumo, Y., Kuzumaki, N., Nakajima, M., et al., 2006. Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. Neuropsychopharmacology 31 (4), 739–750. https://doi.org/10.1038/sj.npp.1300858.

Parent, A.J., Beaudet, N., Beaudry, H., Bergeron, J., Bérubé, P., Drolet, G., et al., 2012. Increased anxiety-like behaviors in rats experiencing chronic inflammatory pain. Behav. Brain Res. 229 (1), 160–167. https://doi.org/10.1016/j.bbr.2012.01.001.

Schwartz, N., Temkin, P., Jurado, S., Lim, B.K., Heifets, B.D., Polepalli, J.S., Malenka, R. C., 2014. Decreased motivation during chronic pain requires long-term depression in the nucleus accumbens. Science 345 (6196), 535–542. https://doi.org/10.1126/science.1253994.

Serafini, R.A., Pryce, K.D., Zachariou, V., 2020. The mesolimbic dopamine system in chronic pain and associated affective comorbidities. Biol. Psychiatry 87 (1), 64–73. https://doi.org/10.1016/j.biopsych.2019.10.018.

Shen, R.Y., 2003. Ethanol withdrawal reduces the number of spontaneously active ventral tegmental area dopamine neurons in conscious animals. J. Pharmacol. Exp. Therapeut. 307 (2), 566–572. https://doi.org/10.1124/jpet.103.053371.

Siciliano, C.A., Calipari, E.S., Yorgason, J.T., Lovinger, D.M., Mateo, Y., Jimenez, V.A., et al., 2016. Increased presynaptic regulation of dopamine neurotransmission in the nucleus accumbens core following chronic ethanol self-administration in female macaques. Psychopharmacology 233, 1435–1443. https://doi.org/10.1007/s00213-016-4239-4.

Strickley, R.G., 2004. Solubilizing excipients in oral and injectable formulations. Pharm. Res. 21 (2), 201–230. https://doi.org/10.1023/b:pham.0000016235.32639.23. PMID: 15032302.

Weiss, F., Parsons, L.H., Schulteis, G., Hyytiä, P., Lorang, M.T., Bloom, F.E., Koob, G.F., 1996. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. J. Neurosci. 16 (10), 3474–3485. https://doi.org/10.1523/JNEUROSCI.16-10-03474.1996.

Yoshimoto, K., Ueda, S., Kato, B., Takeuchi, Y., Kawai, Y., Noritake, K., Yasuhara, M., 2000. Alcohol enhances characteristic releases of dopamine and serotonin in the central nucleus of the amygdala. Neurochem. Int. 37 (4), 369–376. https://doi.org/10.1016/s0197-0186(00)00037-1.

Yu, W., Hwa, L.S., Makhijani, V.H., Besheer, J., Kash, T.L., 2019. Chronic inflammatory pain drives alcohol drinking in a sex-dependent manner for C57BL/6J mice. Alcohol 77, 135–145. https://doi.org/10.1016/j.alcohol.2018.10.002.

Zahm, D.S., 1999. Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Ann. N. Y. Acad. Sci. 877 (1), 113–128. https://doi.org/10.1111/j.1749-6632.1999.tb09264.x.

Zhao, Z., Kim, S.C., Zhao, R., Wu, Y., Zhang, J., Liu, H., et al., 2015. The tegmental–accumbal dopaminergic system mediates the anxiolytic effect of acupuncture during ethanol withdrawal. Neurosci. Lett. 597, 143–148. https://doi.org/10.1016/j.neulet.2015.04.045.