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Title: Male and female rats show opiate withdrawal-induced place aversion and extinction in a Y-maze paradigm

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Abstract

Gender differences have been observed in the vulnerability to drug abuse and in the different stages of the addictive process. In opiate dependence, differences between sexes have been shown in humans and laboratory animals in various phases of opiate addiction, especially in withdrawal-associated negative affective states. Using a Y-maze conditioned place aversion paradigm, we investigated potential sex differences in the expression and extinction of the aversive memory of precipitated opiate withdrawal state in morphine-dependent rats. No significant difference between sexes was observed in the occurrence of withdrawal signs following naloxone injection during conditioning. Moreover, opiate withdrawal memory expression and extinction following repeated testing was demonstrated in both male and female rats, with no significant differences between sexes. Finally, we report spontaneous recovery following extinction of opiate withdrawal memory. Altogether these data provide further evidence that persistent withdrawal-related memories may be strong drivers of opiate dependence, and demonstrate that both males and females can be

used in experimental rodent cohorts to better understand opiate-related effects, reward, aversive state of withdrawal, abstinence and relapse.

Keywords

Opiate dependence / Withdrawal aversive state / Sex variable / Place aversion / Recovery

1. Introduction

Sex as a biological variable is an increasing issue in neuroscience since the under-representation of female subjects in animal research may have led to biased data [1]. In the field of addiction, several studies have revealed clear gender differences in vulnerability to drug abuse and in the different stages of the addictive process (e.g. [2,3]. This has been especially analyzed in the context of the world wide opioid crisis, as women are more likely to be prescribed opioids in the general population and may become addicted more quickly than men [4]. A recent study has shown that the number of women using heroin has increased at a faster rate than for men, despite a global decrease in non-medical opioid use for both sexes [5]. Also, women experience more aversive effects during withdrawal and are more likely to relapse [6]. It is therefore crucial that preclinical research on drug addiction considers these sex differences when studying the various stages of addiction such as binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation [for review 7]

In opiate addiction research, sex differences have been observed in preclinical rodent models of opioid reward [for review 8]. Specifically, females show more opioid reward than males at low doses [9], and acquire morphine and heroin self-administration faster and with higher motivation than males (e.g. [10,11]). Differences between males and females have also been observed in the expression of somatic signs of withdrawal in rats and mice depending on drug regimen, duration, and strain or model used ([12–16]. In particular,

morphine-dependent female rats showed lower withdrawal score than males following spontaneous withdrawal from either repeated morphine injection or morphine pellet implantation, but these differences were no longer observed when withdrawal was precipitated by the injection of naloxone [12].

Conditioned place aversion (CPA) is a particularly sensitive paradigm to measure the aversive state of withdrawal and its memory. Indeed, CPA can be observed even when withdrawal is precipitated with low doses of naloxone that do not induced any overt somatic signs [17]. The few studies that have assessed sex differences in this paradigm have produced conflicting data depending on the model in mice or in specific outbred lines of rat [16,18]. However, it has been shown that, despite an initial similar CPA response to naloxone-precipitated withdrawal, male mice extinguish their aversion later than females [18].

The aim of the current study was to investigate potential sex differences in the expression and extinction of the aversive memory of opiate withdrawal state in morphine-dependent rats. To achieve this, we used a Y-maze CPA paradigm which allows unbiased and segregated measures of aversion, but also safety/preference and neutrality [19]. This paradigm appears highly suitable as sex differences in threat/safety discriminations have been reported in other aversive conditioning paradigms [20,21].

2. Material and Methods

2.1. Animals

Thirty-two Sprague-Dawley CD rats (16 males and 16 females, Charles River Laboratories, France) were individually housed under an inverted 12-h light/dark cycle at $21\pm 2^{\circ}\text{C}$ with food and water available *ad libitum*. Animals were handled daily for two weeks.

Males and females were 14 weeks old at the beginning of the experiment, and weight varied from 250 to 300 g for females and from 400 to 450 g for males. Surgical and experimental procedures were performed in accordance with the European Community's Council Directive (EU Directive 2010/63/EU86) for the care and use of laboratory animals. The present experiment was approved by the local Animal care and Use committee and the French "Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche" (APAFIS#2661-20 15110914459121)

2. 2. Behavioral paradigm: opiate withdrawal conditioned place aversion

Morphine dependence (n=16, 8 males, 8 females) was induced by subcutaneous implantation of two morphine pellets (75 mg of morphine base per pellet; National Institute on Drug Abuse, Bethesda, MD), as previously described [17,19]. One female had to be excluded from the study following morphine-pellet implantation because of respiratory depression. Placebo pellets were used as controls (n=16, 8 males, 8 females). Withdrawal was precipitated by a naloxone injection (15µg/kg s.c.) which induced a strong aversive state with mild somatic signs only in morphine treated rats [17]. Place aversion conditioning (Figure 1A) occurred in an unbiased Y-maze with three compartments [19]. Four days after morphine- or placebo-pellets implantation (day 0), rats underwent a pre-conditioning phase (day 4) during which they freely explored the Y-maze for 20 min. The time spent in each compartment was recorded and predefined exclusion criteria were applied. Specifically, rats were excluded if they displayed spontaneous aversion (less than 17% of the session time) or preference (more than 48% of the session time) for one of the arms (e.g. [22,23]). Based on these criteria, no rats were excluded in the present study. Then, for each rat, the two compartments with the most similar time allotments were assigned to naloxone (CS+) or saline (CS-). The remaining compartment was considered as neutral.

From day 5 to 10, animals underwent conditioning during which all rats received a saline (Sal) injection on days 5, 7 and 9, and a naloxone injection (Nal, 15µg/kg) on alternate days

(6, 8 and 10) immediately before being confined in one compartment of the Y-maze for 20 min. After each conditioning session, the occurrence of some typical withdrawal signs was quantified to compute a score of withdrawal state [see 17,19,24,25]. In the present study we focused on the following withdrawal-related somatic parameters: number (n) of defecations ($n=0$; $<3=1$; $\geq 3=2$) and diarrhea ($n=0$, $n>0=n+1$), and presence of escape behavior/irritability/vocalizations ($no=0$, $yes=2$). The testing phase consisted in a free exploration of the Y-maze for 20 min, during which the time spent in each compartment was recorded. The initial test was performed at 24hrs following the last conditioning session (day 11), as described previously [19,22]. A place aversion/preference score was calculated as the difference between the time spent in the same compartment during test minus pre-test.

Four days after the test, male and female morphine groups underwent an extinction procedure [26] with twice daily test sessions (one in the morning, one in the afternoon) for eight days ($n=16$ tests in extinction). Results were expressed as the mean of the two daily sessions over eight days (Ext 1 to Ext 8). A remote test (RT) was performed three weeks after the last session of extinction to evaluate potential spontaneous recovery. To assess how animals perceived Naloxone (Nal) versus Saline (Sal) compartments, a discrimination index (Di) was calculated as $(\text{Time in Nal}) - (\text{Time in Sal}) / (\text{Time in Nal}) + (\text{Time in Sal})$. Negative values indicated that animals spent more time in the saline-paired compartment than in the naloxone-paired compartment as compared to pre-test for which the index was close to zero (no discrimination).

2.3. Statistics

All statistical analyses were performed with Prism 9.3.0 (GraphPad Software, San Diego, USA). Normality distribution was verified using the Kolmogorov-Smirnov test. Withdrawal scores during conditioning for all groups were analyzed with a three-way repeated measures ANOVA assessing Treatment (placebo vs morphine), Sex (males vs females) and Session effects. Dunnett's multiple comparison tests were used to compare the various sessions to a reference value (Sal1) for each sex. A two-tailed paired t-test was used for time comparison

between Test and Pre-test for each compartment. Aversion/preference score values (i.e. time in test minus time in pre-test) of the compartments between experimental groups were analysed using two-way repeated measures ANOVA and Sidak's multiple comparisons tests to compare males and females within each treatment groups (placebo or morphine). An unpaired t-test was used to compare the discrimination index between males and females at test. Extinction data were analyzed with two-way repeated measures ANOVA, with Dunnett's multiple comparison tests to compare the various sessions with a reference value for each sex (pre-test or test), and Sidak's multiple comparison post hoc tests to compare male and females when appropriate. All ANOVAs were performed with the Geisser-Greenhouse correction for potential violation of sphericity recommended for multiple repeated measures.

3. Results

3.1. Expression of conditioned place aversion associated with withdrawal in males and females

Figure 1 shows that there were no significant differences between males and females in the occurrence of withdrawal signs during conditioning or in the expression of aversion during test. A three-way repeated measures ANOVA assessing Treatment (morphine vs placebo), Sex (males vs females) and Sessions showed a significant effect of morphine ($F_{(1, 27)}=40.23$, $p<0.0001$) and treatment x sessions interaction ($F_{(5, 35)}=20.29$, $p<0.0001$), but no effect of Sex ($F_{(1, 27)}=0.5694$, $p=0.457$). Figure 1B shows that in both male and female morphine-dependent rats (solid lines), naloxone injections (Nal1, Nal2, Nal3) reliably precipitated behavioral signs of withdrawal, which are absent following saline injections (Sal1, Sal2, Sal3). In placebo rats, naloxone injection did not induce any behavioral state of withdrawal in males or females (Figure 1B, dotted lines). For the morphine-treated groups, two-way repeated measures ANOVA (sessions vs sex) showed a significant difference between sessions but no effect of sex or interaction between these factors (Sessions: $F_{(3, 002)}$,

$39.03)=22.28$, $p<0.0001$; Sex: $F_{(1, 13)}=0.01064$, $p=0.9194$; Interaction: $F_{(5, 65)}= 0.5144$, $p=0.7644$). Post-hoc Dunnett's multiple comparison tests showed that all naloxone sessions induced significant withdrawal scores as compared to the first saline injection (Sal1, Figure 1B, $p<0.05$ for all naloxone sessions in both sexes).

Figure 1C and 1D show the behavioral scores for each compartment for the test and pre-test. In placebo controls (Figure 1C), males and females showed no aversion or preference towards any of the compartment. In morphine-dependent rats (Figure 1D), both males and females displayed an aversion for the naloxone-paired compartment (Paired t-test; two-tailed, **** $p<0.0001$ for males, *** $p=0.0004$ for females). Only females showed a significant preference for the saline-paired compartment (Paired t-test; two-tailed, ** $p=0.0037$ for females). No significant differences were found in scores for the neutral compartment in males or females. To determine if males and females behave similarly towards the various compartments, a two-way repeated measures ANOVA (compartments vs sex) was performed for the morphine-treated groups. The results showed a significant difference between compartments but no effect of sex or interaction between these factors (Compartments: $F_{(1,241, 16,14)}=16.18$, $p=0.0005$; Sex: $F_{(1, 13)}=0.6285$, $p=0.4421$; Interaction: $F_{(2, 26)}= 2.509$, $p=0.1009$). Furthermore, to measure how animals reliably differentiate the aversive (CS+ naloxone-paired) compartment from the safe one (CS- saline-paired), we compute a discrimination index (Di). As expected, the Di was significantly different between test and pre-test (Figure 1E, two-tailed Paired t-test, ** $p=0.0011$ for males; *** $p=0.0005$ for females). However, no significant difference in Di was observed between males and females (two-tailed Unpaired t-test, ns $p=0.7240$).

3.2. Extinction of the behavioral response in males and females

We next wanted to examine the extinction of these emotional responses. To do so, four days after the test, extinction of conditioned responding was assessed through repeated test sessions, twice daily for 8 days (16 extinction sessions). One final remote test (RT) was also

conducted three weeks after the final extinction session to evaluate potential spontaneous recovery. For extinction sessions, data were represented as the mean of the two daily sessions over the eight days (Ext 1 to Ext 8).

Figure 2 shows the evolution of the time spent in each compartment, as well as the discrimination index (Di), at pre-test, over daily extinction sessions (Ext 1 to Ext 8), and during the remote test (RT). A two-way repeated measures ANOVA was performed to detect potential differences between males and females following repeated testing in each compartment (times and discrimination index related to pre-test). The results demonstrated an overall effect of session for both naloxone (CS+) and saline (CS-) paired compartments ($F_{(4.353, 56.59)}=8.633$, **** $p=0.0011$ for naloxone, $F_{(4.092, 53.201)}=5.254$, ** $p=0.0011$ for saline), as well as for the discrimination index ($F_{(4.313, 56.07)}=10.07$, **** $p<0.0001$). These data showed that the initial aversion for the naloxone compartment progressively decreased across the extinction sessions (Figure 2A), which was accompanied by a progressive decrease of their preference for the saline context (Figure 2B) and a loss of significant naloxone/saline discrimination (Figure 2D). As expected time spent in the neutral compartment was similar across sessions (Figure 2C, $F_{(4.757, 61.84)}=1.515$, $p=0.2007$). No difference was found between males and females for the time spent in each compartment or for the discrimination index (see Table 1 for statistical details).

Since no statistical differences between males and females were detected, the data from both sexes was pooled and additional analyses were conducted on this pooled data to better investigate extinction and potential spontaneous recovery following the remote test (Figure 3). Three-way repeated measures ANOVA (compartment vs session) demonstrated a significant difference between compartments ($F_{(2, 42)}=13.13$, $p<0.0001$) and a significant compartment x session interaction ($F_{(20, 420)}= 6.423$, $p<0.0001$). Post hoc Dunnett's multiple comparison showed that aversion for the naloxone paired compartment started to extinguish after 9 to 10 test sessions (Ext 5) but recovered at the remote test (** $p=0.0056$). We also computed the discrimination index (Di, Figure 3B) on pooled data and a one-way repeated measures

ANOVA revealed a significant difference between sessions ($F_{(4.407, 61.69)}=10.36$, **** $p<0.0001$). Post hoc Dunnett's multiple comparisons further demonstrated that animals no longer discriminated between naloxone and saline compartments between Ext 5 and Ext 8. Interestingly, following this extinction, spontaneous recovery of the behavioral response occurred when re tested 3 weeks later (RT, * $p=0.0171$), but aversion was lower than during the initial test (####, $p<0.0001$ between test and RT).

4. Discussion

Affective signs of withdrawal are key drivers of opiate dependence supporting the role of negative reinforcement in the maintenance of opiate dependence as well as the role of conditioned withdrawal in relapse [26,27]. Interestingly, when compared to men, women experience more aversive effects during withdrawal from opiates and are more likely to relapse [6]. Here, we find no evidence that opiate withdrawal memory expression and extinction are significantly different in male and female morphine-dependent Sprague-Dawley rats in a Y-maze paradigm.

4.1. Acquisition and expression of conditioned place aversion

We first measured the occurrence of withdrawal signs following naloxone injection during each conditioning session. Indeed, while some differences have been reported between males and females in the expression of spontaneous opiate withdrawal signs in rats and mice depending on drug regimen and duration (e.g. [12,14]), naloxone-precipitated withdrawal is likely to mask these sex differences [12]. Accordingly, we found here that withdrawal scores measured during conditioning did not differ between males and females following morphine pellet implantation, in which the aversive state of withdrawal was precipitated by naloxone injection. We also further confirm the absence of withdrawal signs and conditioned place aversion in both non-dependent (placebo) male and female rats, as previously described [17,19,25].

Regarding the expression of conditioned place aversion to naloxone-precipitated withdrawal, we show no significant differences between male and female rats. This is consistent with previous data obtained in mice showing no sex differences in conditioned place aversion using a similar paradigm [18]. The Y-maze paradigm also allows us to assess safety coding, defined as an avoidance of the withdrawal-paired compartment and a preference for the saline-paired compartment [19,24,28]. We found here that females also showed a significant preference for the saline compartment. However, this preference was not significant in males in the present study probably due to inter-individual differences. Indeed such preference for the saline compartment has been consistently observed in males in previous studies –including ours- using the same dose of naloxone to precipitate the aversive state of withdrawal [19,24,28]. Moreover, analysis of the discrimination index indicated that both males and females display strong and similar discrimination between naloxone (aversive) and saline (safe) compartments. Altogether, these data show that, in our experimental conditions, female rats are equally sensitive to naloxone as males, and appear to display similar opiate withdrawal memory expression, and aversive/safety discrimination.

4.2. Extinction and spontaneous recovery

Using the Y-maze CPA, we have previously shown that aversive opiate withdrawal memory is very sensitive and persistent [29,30]. In this paradigm, extinction of the aversive memory can be assessed either through repeated testing or through repeated confinements in the withdrawal-and saline-paired compartments before test [26,31]. Unlike the two-compartment CPA paradigm that has classically been used [31–33], the Y-maze paradigm allows segregated measures of aversion and safety/preference and neutrality [19]. Therefore, extinction training through repeated testing can differentiate between active avoidance (escaping the aversive context) and passive avoidance (staying in an identified safe context). Our results show that extinction of the aversion for the withdrawal-paired compartment and of

the aversion/safety discrimination occurred after 5 days (Ext 5; i.e. 9-10 sessions) in both males and females. This is consistent with some data obtained in rats and mice showing, in a two-compartment paradigm, that extinction occurred following at least 6 consecutive testing sessions [18,33,34], and provides further support that opiate withdrawal produces a long-lasting aversive memory [30,35]. Also, the persistence of the CPA behavioral response may also depend upon the extinction procedure [26]. Indeed, extinction through reconditioning may occur more rapidly in a classical two-compartments CPA paradigm [31,32,36].

Interestingly, no significant difference was found here as a function of sex. This result differs from one study in mice showing that opiate withdrawal CPA extinction is delayed in males as compared to females [18]. Also, sex differences have been described in several other aversive conditioning paradigms [37,38]. For example, females rats show better fear/safety discrimination [20], but may generalize with extended training due to impaired safety learning [21]. Thus additional studies are needed to better disentangle potential sex differences in the extinction of opiate withdrawal related memories, in comparison with other types of aversive conditioning. Despite the absence of significant sex effect nor interaction for all various the parameters analysed in the present study (withdrawal score, aversion/preference scores, times in compartments or discrimination index during extinction), we cannot fully exclude that we might have missed some subtle differences between males and females due to a lack of power. However, all our data more likely suggest a significant overlap with many females showing similar behavioural responses to many males, in accordance with the idea of a continuum being more frequent than sexual dimorphisms in many studies as reviewed [1].

Spontaneous recovery refers to a reappearance of an extinguished conditioned response with the passage of time in the absence of any further explicit training [26,39]. In a Y-maze paradigm, in absence of extinction, it has been shown that the aversion for the opiate withdrawal paired compartment persists with time [30]. However, the data presented here are the first to report spontaneous recovery following extinction of opiate withdrawal memory in a

CPA paradigm. Our results indicate that, like other aversive memories, extinction of opiate withdrawal related memories does not erase the original memory as the expression of this memory can recover with time [26,39]. The behavioural and neurobiological mechanisms of such recovery remain to be established in the present paradigm.

5. Conclusion

In summary, the present data demonstrate opiate withdrawal memory expression and extinction in both males and females in a Y-maze CPA paradigm, with no significant difference between sexes. Also, following extinction, spontaneous recovery of the aversive memory occurs. These data further highlight the persistence of withdrawal-related memories in opiate dependence, which may drive drug seeking and relapse at long term after abstinence. Overall, very few studies have systematically assessed sex differences in opiate withdrawal memory. The present data, obtained in Sprague Dawley rats, and in a specific experimental model, does not imply that sex differences may not exist in other models or strain of rats or mice, and for specific features of opiate dependence and withdrawal [see 12,14,16,18]. Thus, the present study supports the need for sex diversity in experimental rodent cohorts to better understand the various components of opiate dependence including rewarding effects, aversive state of withdrawal, abstinence and relapse. More generally increased awareness of the importance of including females in preclinical research will be to the benefit of many fields in neuropsychiatric research.

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CRedit authorship contribution statement

M.L. Fournier, A. Faugère, O. Barba-Vila: Investigation;

M.L. Fournier, C. Le Moine: Formal analysis;

C. Le Moine: Writing - Original Draft;

M.L. Fournier, O. Barba-Vila: Writing - Review & Editing. All authors have accepted the final manuscript before submission.

Table 1: Two-Way RM ANOVA with Geisser-Greenhouse correction

Extinction		F value way	P value	Significance
Naloxone compartment	Sex	F (1, 13) = 0.00047.	0.9831	no
	Sessions	F (4.353, 56.59) = 8.633	0<0.0001	****
	Interaction	F (10, 130) = 0.9445	0.4952	no
Saline Compartment	Sex	F (1, 13) = 0.04212	0.8406	no
	Sessions	F (4.092, 53.20) = 5.254	0.0011	**
	Interaction	F (10, 130) = 0.9998	0.4472	no
Neutral compartment	Sex	F (1, 13) = 0.01670	0.8992	no
	Sessions	F (4.757, 61.84) = 1.515	0.2007	no
	Interaction	F (10, 130) = 1.777	0.0708	no
Discrimination index	Sex	F (1, 13) = 0.001831	0.9665	no
	Sessions	F (4.313, 56.07) = 10.07	<0.0001	****
	Interaction	F (10, 130) = 0.5633	0.8412	no

Figure legends

Figure 1: Acquisition and expression of opiate withdrawal conditioning in male and female rats (A) Conditioned place aversion paradigm. Conditioning occurred in an unbiased Y-maze. Four days after morphine or placebo pellet implantation, rats underwent a pre-test during which they freely explored the Y-maze for 20 min. During conditioning, rats received saline injections (days 5, 7, and 9) or naloxone 15µg/kg (days 6, 8, and 10) and were confined to a given compartment for 20 min. The test phases consisted in a free exploration of the Y-maze for 20 min starting 24hrs after the last conditioning session. The time spent in each compartment was recorded, and a place aversion score was calculated as the difference between the percentage of time spent in the same compartment during test minus pre-test. **(B)** Evaluation of the withdrawal score during conditioning in both morphine and placebo treated rats. After each conditioning session, the occurrence of some withdrawal signs (defecation, diarrhea, escape, and vocalizations) were measured to allow computation of a score of withdrawal state. As expected, naloxone injection (15µg/kg) induced overt signs of withdrawal only in morphine-dependent rats and only during naloxone pairing sessions (Nal1, Nal2, and Nal3) as compared to the first saline injection (Sal1), with no significant differences between males and females (see results section for detailed statistics). Black = males, grey = females; solid lines = morphine groups, dotted lines = placebo groups. **(C, D)** Aversion and/or preference scores during test for each compartment. (Naloxone CS+ dotted, Saline CS- plain, Neutral N stripes; grey for males, white for females) between test and pre-test. In placebo controls (Figure 1C), neither males nor females showed any aversion or preference for any compartment. In morphine-dependent rats (Figure 1D), both males and females displayed an aversion for the naloxone-paired compartment (Paired t-test; two-tailed, **** $p < 0.0001$ for males, *** $p = 0.0004$ for females). Only females showed a significant preference for the saline-paired compartment (Paired-t-rank test; two-tailed, ** $p = 0.0037$ for females). No significant differences were found for the neutral compartment. Two-way repeated measure (RM) ANOVA (compartments vs sex) for the morphine-treated groups

showed no effect of sex nor interaction (Compartments: $F_{(1,241, 16.14)}=16,18$, $p=0.0005$; Sex: $F_{(1, 13)}=0,6285$, $p=0,4421$; Interaction : $F_{(2, 26)}= 2,509$, $p=0.1009$). **(E)** Discrimination index between test and pre-test. Males (grey bar) and females (white bar) displayed identical discrimination between naloxone- and saline-paired compartment at test (two-tailed Paired t-test between test and pre-test, ** $p=0.0011$ for males; *** $p=0.0005$ for females; two-tailed Unpaired t-test between males and females, ns $p=0.7240$).

Figure 2: Extinction of the expression of opiate withdrawal memory with repeated testing in males and females

(A, B, C) Evolution of time spent in naloxone/CS+ **(A)**, saline/CS- **(B)** and neutral compartments **(C)**. Males: black lines and circles, Females: grey lines and circles. Data from the two daily sessions were averaged for each day (Ext 1 to Ext 8). For naloxone and saline compartments, two-way RM ANOVA with GGC (session vs sex) showed a session effect but no effect of sex nor interaction between these factors (see Table 1 for details). Time spent in the neutral compartment was similar across sessions and for both sexes. **(D)** Evolution of the discrimination index (Di) calculated as $(\text{Time in Nal}) - (\text{Time in Sal}) / (\text{Time in Nal}) + (\text{Time in Sal})$. Two-way RM ANOVA with GGC (session vs sex) again showed an effect of session but no effect of sex or interaction between these factors. ***S and **S = main session effect for naloxone and saline compartments respectively; ****S: main session effect for Di.

Figure 3: Extinction and spontaneous recovery on pooled data

(A) Evolution of time spent in each compartment naloxone (black circles), saline (black triangles) and neutral compartments (grey circles). Extinction of the aversion for the naloxone paired compartment began at Ext 5 but recovered at RT, while preference for saline was no longer significant from Ext 2. RT: remote test 3 weeks following the end of extinction.

(B) Discrimination index showed that rats lose their capacity to discriminate between naloxone and saline compartments from Ext 5 to Ext 8 following twice daily repeated testing. However, spontaneous recovery of the behavioral response occurred when re tested 3 weeks following the last extinction test (RT, $p=0.0171$), but with a lower intensity than the initial test (* $p<0.05$ vs Pre-test, ####, $p<0.0001$ vs test).

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Fig 1

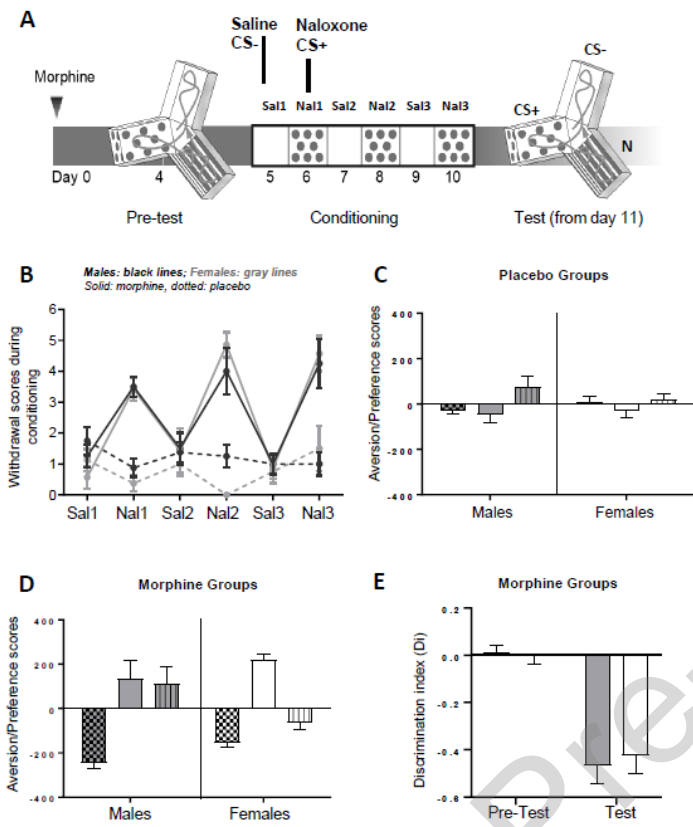


Fig 2

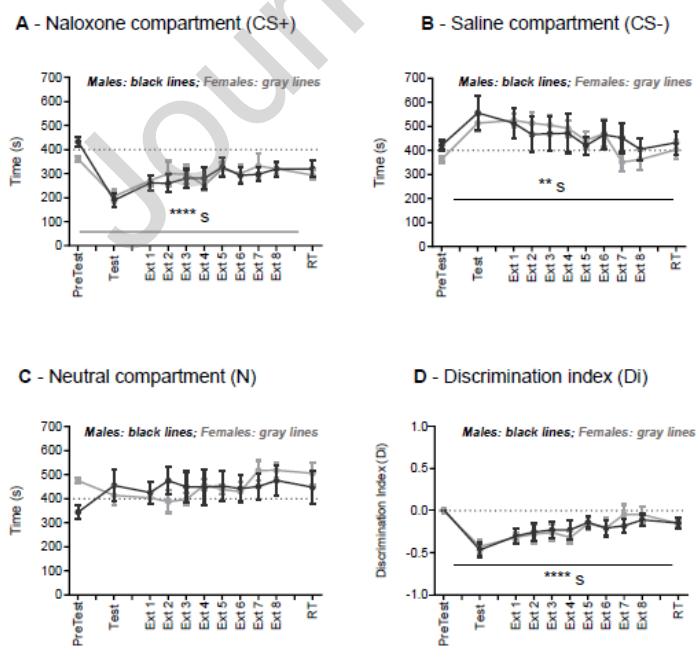
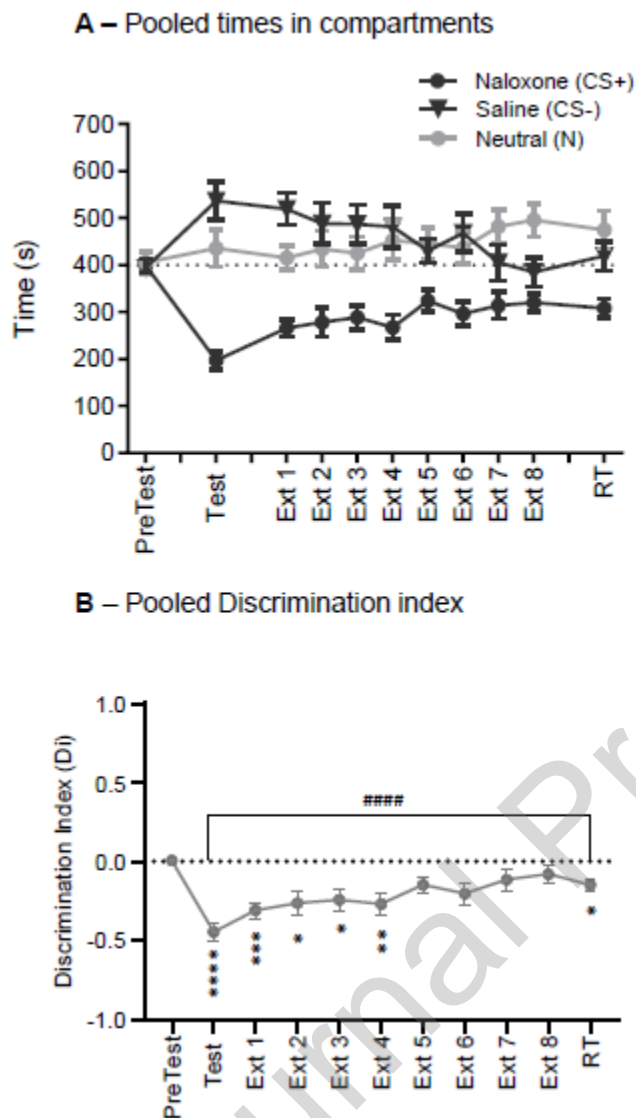


Fig 3



Highlights

- ✓ Studying sex differences is crucial in opiate dependence and withdrawal.
- ✓ Opiate withdrawal conditioned place aversion was analyzed in male and female rats.
- ✓ A Y-maze paradigm was used to assess both aversion and safety learning
- ✓ No sex differences were observed in expression and extinction of aversion.