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Short-term stress significantly decreases morphine analgesia in trigeminal but not in spinal innervated areas in rats

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ABSTRACT

Plenty information exists regarding the effects of chronic stress, although few data exist on the effects of short-lasting stressors, which would mimic daily challenges. Differences in craniofacial and spinal nociception have been observed, thus those observations obtained in spinally innervated areas cannot be directly applied to the orofacial region. Although, opioids are considered amongst the most effective analgesics, their use is sometimes hampered by the constipation they induce. Thus, our aims were to study if a short-lasting stressor, forced swim stress (FSS), modifies nociception, morphine antinociception and constipation in rats. Animals were submitted to 10–20 min of FSS for three days, nociception and gastrointestinal transit were studied 24 h after the last swimming session. Nociception and morphine (0.6–5 mg/kg) antinociception were evaluated in the formalin and hypertonic saline tests in the orofacial area and limbs. Morphine-induced modifications in the GI transit were studied through radiographic techniques. Naloxone was administered, before each swimming session, to analyse the involvement of the endogenous opioid system on the effect of stress. Overall, stress did not alter nociception, although interestingly it reduced the effect of morphine in the orofacial tests and in the inflammatory phase of the formalin tests. Naloxone antagonized the effect of stress and normalized the effect of morphine. Stress did not modify the constipation induced by morphine. Opioid treatment may be less effective under a stressful situation, whilst adverse effects, such as constipation, are maintained. The prevention of stress may improve the level of opioid analgesia.

1. Introduction

Habituation to repeated stressors is an adaptive mechanism which can limit the occurrence and severity of stress-related symptoms [1,2]. However, these adaptive responses can become dysregulated and result in disease [3].

During the past decades there has been a great interest in understanding the interactions between stress and pain. Depending on the nature, duration and intensity of the stressor, stress can exert modulatory influences typified by either a reduction or exacerbation of pain. Thus, exposure to an acute, strong, intense stressor produces stress-induced analgesia, while repeated or chronic exposure to physical or psychological stressors produces stress-induced hyperalgesia (SIH) in humans (for review, see [4,5].

To study the effects of repeated stressors in nociception in preclinical studies, different stress models exist in the literature. Most of them, such as chronic social defeat stress [6,7] and chronic restraint stress [8] could mimic chronic stressors and they are sufficiently long lasting and/or intense to induce changes in nociception. On the contrary, very little attention has been paid to short-lived stressors, which could mimic the human daily stressors, such as an unexpected work deadline or traffic jam, that are minor challenges, yet they occur very frequently and

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independent of chronic stressors [9]. Thus, little is known in preclinical studies on how they can affect normal pain processing, and in this regard, forced swimming for three consecutive days has previously been used as a "short-term stressor" [10].

Muscular pain is one of the most prevalent pain disorders, and among the main causes of disability and health consuming resources worldwide. It has long been known that stress has an important impact on muscle pain, for example, in non-specific low back pain or the development of temporomandibular disorders [11–13]. Despite this, no study has investigated the effects of short-term stress on muscle pain.

Of all the pharmacological options for the treatment of pain, opioids are considered amongst the most effective, although their use is sometimes hampered by their side effects, being opioid induced constipation the most common one, to which tolerance rarely develops [14].

Previous studies have demonstrated that important differences exist in pain processing between the craniofacial and spinal systems, both regarding sensitivity [15] or pain processing [16,17]. Therefore, the mechanisms of craniofacial pain are unique and cannot be considered identical to those of spinal processing [18]. With regards to these differences, we have previously demonstrated that the analgesic efficacy of some opioids was greater when pain is located in the orofacial region [15,19].

Thus, considering the frequency of daily stressors, the importance that stress plays in muscle pain and the differences that exist between craniofacial and spinal innervation in the nociceptive processing. Our aims are to study the effect of forced swimming stress (FSS) in:

- Two models of nociception in the orofacial and limb areas
- The morphine-induced antinociception and
- The GI transit modifications induced by morphine

2. Methods

2.1. Ethical statement

All experimental animal procedures were carried out according to a protocol approved by the Research Ethics Committee of Universidad Rey Juan Carlos (Ref. 0910201811618) and, following the guidelines for the Care and Use of Laboratory Animals of the European Community (European Directive 2010/63/EU) and those of the International Association for the Study of Pain on ethical standards for investigation in animals [20].

2.2. Animals

Male Wistar rats (250–300 g) (Charles River Laboratories, France) were used. The animals were housed six per cage in transparent cages (60 ×40×20 cm) with wood shavings as bedding, and with free access to food and water. The test room was maintained at a temperature of 23 ± 1 °C, humidity of 60%, and had a 12 h light/dark cycle (08:00–20:00 h); animals were habituated in the test room for at least 5 days before experimentation.

2.3. Drugs

Morphine sulfate (Alcaliber, Spain) and naloxone (Sigma-Aldrich, Spain), an opioid receptor agonist and antagonist, respectively, were dissolved in 0.9% saline solution. All solutions were freshly prepared before each experiment.

The formalin solution was prepared from a commercially available stock (an aqueous solution of 37% formaldehyde, Panreac Química, S.A.U.), further diluted in saline to reach a final concentration of 2.5%.

The barium sulfate suspension used as the contrast medium for the radiographic study was suspended in tap water (2 g/ml, t = 22 °C; Barigraph ® AD, Juste SAQF, Madrid, Spain).

2.4. Forced swim stress procedures

In order to induce stress, the repeated FSS paradigm was used as described previously [21]. Rats were placed in a cylinder (40 cm in diameter and 50 cm in height) filled with clean tap water (24 −26 °C) up to a height of 30 cm. Rats were submitted to forced swimming once a day for three consecutive days; the first day for 10 min and the subsequent two days for 20 min. After each session, rats were dried up with a towel and placed back into their cages. The rats of the control groups remained undisturbed in their cages.

2.5. Behavioural assessments

2.5.1. Anxiety assessment

To test if the FSS paradigm produced an anxiety-like behaviour, the elevated plus-maze was used [22]; the maze is a plus-shaped platform with two opposite open arms (50.8 cm × 10.2 cm) and two closed arms (50.8 cm × 10.2 cm × 40.6 cm), which are elevated 72.4 cm above the floor. Each animal was placed in the center of the maze and allowed to explore it for five min. The experiment was recorded, and the time (s) spent by each animal in the closed and open arms was counted. The entries and the total time spent into open arms are important indicators to assess the anxiety of animals.

2.5.2. Locomotor activity

The infrared beam-based activity meter (Cibertec, Spain) was used to determine any possible alteration of the spontaneous locomotor activity induced by morphine in control and FSS rats. For such purpose, animals were individually placed in separate photocell activity chambers (55 cm × 40 cm; spacing between beams 3 cm) [23] and the number of crosses (interruptions of photocell beams) was recorded over a 30 min period. The effect of morphine was compared with that of the vehicle, and the FSS rat group with the control rats.

2.5.3. Nociceptive tests

2.5.3.1. Masseter muscle pain. The masseter pain model was used, as previously described [23,24], to assess muscle pain in the orofacial region. Rats were lightly anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the skin over the masseter muscle was carefully shaved. The level of light anesthesia was determined by providing a noxious pinch to the tail or the hind paw with serrated forceps; animals typically respond to these stimuli with an abdominal constriction and with a withdrawal reflex. Experiments were continued only after the animals showed reliable reflex responses to every noxious stimulus.

The ipsilateral hind paw shaking behaviour evoked by hypertonic saline (HS) stimulation of the masseter muscle is accepted as an index of muscle nociception. 100 μl of HS (5% NaCl) were administered into the mid-region of the right masseter muscle, and the total number of shakes was counted by only one researcher to maintain consistency. To do this, the experiments were recorded on video and played back in slow motion.

2.5.3.2. Gastrocnemius muscle pain. The gastrocnemius pain model was used, as previously described [25,26], to assess the muscle pain in the limb region. The injection of 500 μl of HS in the mid-region of the gastrocnemius evokes a nociceptive behaviour, which consists in the withdrawal or flexing of the affected paw. Once HS was injected in the muscle, the animal was kept in a Plexiglas box and two mirrors were positioned underneath and behind it to permit unobstructed viewing of the paw. The time (s) that animals remained with the paw flexed or withdrawn was measured for up to five min.

2.5.3.3. Inflammatory pain

2.5.3.3.1. Orofacial inflammatory pain. The orofacial formalin test

was performed to assess inflammatory pain as described by Burgos et al., 2010 [24]. Briefly, 50 μl of formalin 2.5% were injected subcutaneously (s.c.) in the vibrissal pad, just lateral to the nose. This injection evokes recurrent and persistent episodes of paw strokes directed to the perinasal area (face rubbing), thus the number of seconds the rat spent rubbing the injected area with the ipsilateral fore or hind paw was considered as the nociceptive score.

2.5.3.3.2. Paw inflammatory pain. Paw formalin test was used as previously described [27]. For this, 20 μl of formalin 2.5 % were injected into the plantar surface (s.c.) of the right hind paw. This injection evokes two characteristic behaviours: flinching/shaking and licking/biting of the injected paw. Both behaviours were recorded at the same time and the sum of the number of flinches/shakes plus the time (s) spent licking/biting was considered as the nociceptive response. It is commonly accepted that the combination of these two behaviours provides a better measurement of pain intensity than that of each behaviour alone [7,27].

In both tests (orofacial and paw formalin), one researcher injected formalin, whilst another experimented researcher carefully restrained the rat. After the injection, the animal was immediately placed on a recording chamber. The nociceptive behaviours were measured at three min intervals up to the min 36; this is the period when there are more nociceptive behaviours and furthermore, previous studies have shown that forced swim induces more significant changes in the pain scores within the first 30 min after formalin injection [28,29].

Two periods were considered: an early or acute phase corresponding to acute nociceptive pain that started immediately after the formalin injection until the min 3 (orofacial) or the min 6 (paw); and a late or inflammatory phase, from the min 12 to the min 36 after formalin injection, which corresponds to inflammatory pain. In order to provide an easy representation of the nociception in both phases, these results were transformed into the area under the curve (AUC) using the trapezoidal rule; as a result, a single quantifiable value was given for each phase [30].

2.6. Role of opioid receptors in stress effect on masseter pain

The implication of opioid receptors in the modulatory effect of stress on the antinociceptive effect of morphine was studied in the model of orofacial muscle pain. Naloxone (2 mg/kg) was administered 30 min before each of the three consecutive sessions of forced swimming [25,31].

2.7. Gastrointestinal motor function

Non-invasive radiographic techniques were used to assess the effects of morphine on the GI motor function of control and FSS rats, as previously described [32,33]. Radiographic sessions were carried out 1 h after the administration of morphine or its vehicle. In each session, without using anesthesia, rats were administered *per os* 2.5 ml of a suspension of barium sulfate as a contrast medium, after which plain facial radiographs of the GI tract were taken at different times (immediately after and 1, 2, 4, 6 and 8 h later: times indicated as T0, T1, T2, T4, T6 and T8). A CS2100 (Carestream Dental, Madrid, Spain) digital X-ray apparatus (60 kV, 7 mA) was used. X-rays were recorded on Carestream Dental T-MAT G/RA film (15 ×30 cm) housed in a cassette provided with regular intensifying screen. Films were developed using a Kodak X-omat 2000 automatic processor. The focus distance was manually fixed to 50 ± 1 cm and the exposure time was adjusted to 20 ms. Rats were immobilized in the prone position, by placing them in hand-made, rat-adjustable, transparent plastic tubes. To minimize stress, rats were released from the tubes immediately after each shot and thus, immobilization lasted 1–2 min. GI motility was not significantly altered by habituation of the animals to the recording chamber before the starting of the experiments [32].

The evolution of GI motility in rats was semiquantitatively analysed from radiographic images. Thus, for the different GI regions (stomach, small intestine, caecum and colorectum) the following parameters were considered and scored: percentage of the GI region filled with contrast (0−4); contrast intensity (0−4); contrast homogeneity (0−2) and sharpness of the GI region profile (0−2). After scoring each GI region, a sum of the values obtained for each parameter was made (0–12 points). The analysis of the radiographs was carried out by a trained researcher who was blind to the treatments.

2.8. General procedures and experimental design

Test sessions were carried out between 09:00 and 15:00 h. Animals were randomly assigned to different experimental groups ($N \geq 8$). Separate groups of rats were used for each experimental procedure and treatment and were used only once. The researchers who carried out the experiments were blind to the treatments.

All assays were performed in non-stressed (control) and in stressed rats by FSS and were performed 24 h after the last swimming session in FSS animals [34]. Control animals were left undisturbed in their cages until the day of the experimental procedures (Fig. 1).

Morphine, naloxone and their vehicle were administered intraperitoneally (i.p.) in a volume of 1.5 ml/kg. To test the antinociceptive effect of morphine, doses of 0.6–2.5 mg/kg were used in masseter pain model, 1.25–5 mg/kg in gastrocnemius model and 5 mg/kg in the formalin test. Doses were chosen based on our previous studies [23,25]. The doses of morphine evaluated in the GI motor function assays were 2.5 and 5 mg/kg. The antinociceptive effect of morphine was always evaluated starting 30 min after its administration and compared with that of the vehicle.

2.9. Statistical analysis

All data were analysed and plotted on graphs using the GraphPad Prism 7.0 software (GraphPad Software, San Diego, USA), and expressed as mean ± SEM. The normality of all data was assessed by D'Agostino & Pearson test. Two-way ANOVA test (treatment x stress) followed by Sidak's multiple comparisons test was used to determine statistical significance between morphine treatment and FSS. To analyse the analgesic effect of morphine, comparisons were made with the group of animals treated with vehicle and, to study the effect of stress, comparisons were made with the control group (not subjected to forced swimming).

One-way ANOVA followed by Dunnet's *post hoc* test was used to compare the effect of antagonists. Unpaired *t*-test was used to analyse data from plus-maze test.

Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Behavioural tests

3.1.1. Anxiety assessment

Both control and FSS animals spent more time in closed arms than in open ones. *t*-test showed no significant differences in the time spent in open and closed arms between them (*Closed and open arms*: $p > 0.05$ *vs.* control animals). These data suggest that FSS did not induce an anxiety behaviour (Fig. 2).

3.1.2. Locomotor activity

When animals submitted to FSS were treated with vehicle (Veh), the number of photobeam crosses were reduced when compared to the vehicle control animals ($p < 0.05$). On the other hand, morphine (MF) reversed this reduction in the locomotor activity (MF2.5: $p < 0.01$; MF5: $p < 0.05$ *vs.* Veh) and no differences were found between the control and stressed animals treated with morphine ($p > 0.05$) (Fig. 3).

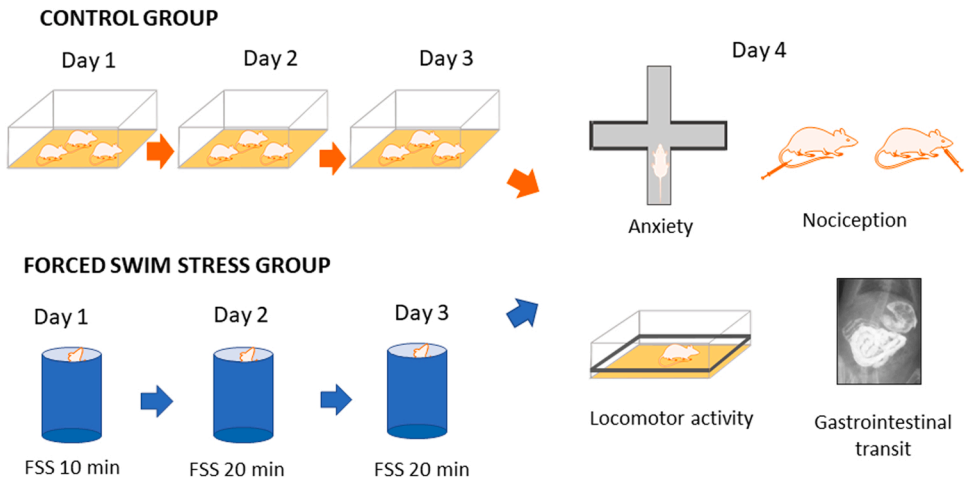

Fig. 1. Experimental protocol. Rats were submitted to force swim stress for three consecutive days or left undisturbed in their cages. On the fourth day, the different assays were performed.

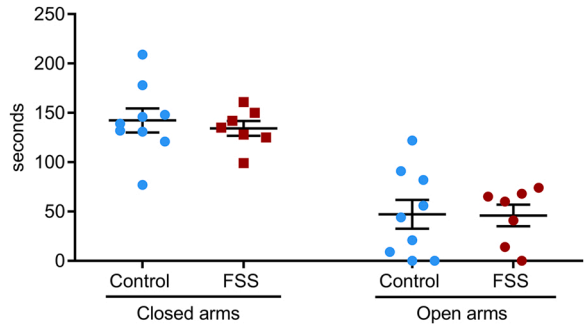

Fig. 2. Influence of forced-swimming stress (FSS) on anxiety-related behaviour in the elevated plus-maze. Each scatterplots represent the mean ± SEM of the time (s) spent in open and closed arms of control and FSS animals. $N \geq 8$.

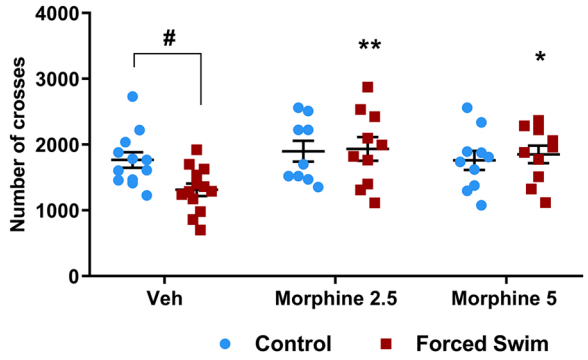

Fig. 3. Effect of stress and morphine on locomotor activity. Each scatterplot shows the number of crosses (mean ± SEM) in the actimeter test, performed by control and stressed animals (FSS) treated with vehicle (Veh) or morphine 2.5 and 5 mg/kg. #$p < 0.05$ *vs.* control (non-stressed animals); * $p < 0.05$, ** $p < 0.01$ *vs.* Veh. Unpaired *t*-test, $N \geq 10$.

3.2. Nociceptive tests

3.2.1. Effect of FSS on muscle pain and on the effect of morphine

3.2.1.1. Masseter pain. Two-way ANOVA test indicated a significant effect of treatment ($F_{(3,84)}= 25.62$, $p < 0.0001$) and stress ($F_{(1,84)}= 12.57$, $p < 0.001$) factors, but not for treatment x stress interaction ($F_{(3,84)}= 1.408$, $p > 0.05$).

Stress did not modify the HS-induced nociceptive behaviour in rats. As expected, morphine (0.6–2.5 mg/kg) reduced the HS induced nociceptive behaviour in control animals (MF0.6: $p < 0.05$; MF1.25 and 2.5: $p < 0.0001$ *vs.* control), but FSS significantly reduced the antinociceptive effect of morphine and only the doses of 1.25 and 2.5 mg/kg had an antinociceptive effect, which was 25% and 36.4%, respectively lower in stressed rats than in control ones ($p < 0.05$ *vs.* control) (Fig. 4 A).

3.2.1.2. Gastrocnemius pain. Two-way ANOVA showed only a significant effect for treatment factor ($F_{(3,73)}= 45.1$, $p < 0.0001$) but not for stress ($F_{(1,73)}= 1.457$, $p > 0.05$) nor treatment x stress interaction ($F_{(3,73)}= 1.178$, $p > 0.05$).

Again, FSS did not modify the nociceptive behaviour in response to HS in vehicle treated rats and morphine reduced the nociceptive behaviour after HS injection (MF1.25: $p < 0.01$; MF2.5 and 5: $p < 0.0001$ *vs.* control). On the contrary as what had happened in the masseter, FSS did not induce a reduced response to morphine when compared to the control animals ($p > 0.05$). (Fig. 4B).

3.2.2. Effect of FSS on inflammatory pain and on the effect of morphine

To reduce the necessary number of animals following ethical considerations and based on the results obtained in the muscle pain experiments and in a previous pilot study (data not shown), a single dose of morphine, 5 mg/kg, was chosen to test its effect in the formalin-induced inflammatory pain model.

3.2.2.1. Orofacial formalin test. The typical biphasic nociceptive response induced by formalin injection, as well as the effect of morphine on control and FSS animals can be seen in Fig. 5A.

Statistical analysis revealed significant effects in the acute phase for treatment ($F_{(1,54)}= 6.283$, $p < 0.05$) and stress ($F_{(1,54)}= 4.199$, $p < 0.05$) factors, but not for interaction between morphine and stress ($F_{(1,54)}= 1.536$, $p > 0.05$). In inflammatory phase, treatment and stress factors had significant effect (treatment: $F_{(1,54)}= 8.646$, $p < 0.01$; stress: $F_{(1,54)}= 5.467$, $p < 0.05$; treatment x stress interaction: $F_{(1,54)}= 2.309$, $p > 0.05$).

Overall, stress did not modify the nociceptive effect of formalin in either of the two phases. Morphine 5 mg/kg reduced the nociceptive response in both the acute ($p < 0.05$, Fig. 5B) and inflammatory ($p < 0.01$, Fig. 5C) phases in control non-stressed animals, whilst in animals submitted to FSS, morphine lost its antinociceptive effect ($p > 0.05$, Fig. 5B and C).

Thus, although FSS did not modify orofacial inflammatory

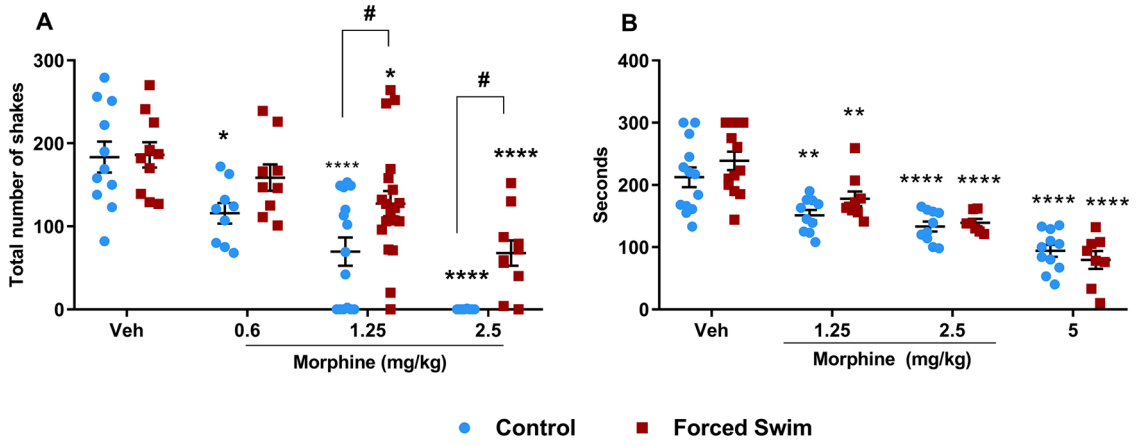

Fig. 4. Nociceptive behaviour induced by HS in the masseter and gastrocnemius muscles. The graphs show the nociceptive responses to HS injection on masseter (A) and gastrocnemius (B) on control rats and rats stressed by forced swimming (FSS), treated with vehicle (Veh) or different doses of morphine. Scatterplots show the mean ± SEM of the total number of hind paw shakes (A) or the time (s) the rat spent with the paw withdrawn or flexed (B). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ *vs.* Veh; #$p < 0.05$ *vs.* non-stressed animals at the same doses of morphine. Two-way ANOVA, followed by Sidak's multiple comparisons *post hoc* test. $N \geq 10$.

nociception, it significantly ($p < 0.05$) reduced by a 26% the antinociceptive effect of morphine in the acute phase and 23% in the inflammatory phase.

3.2.2.2. Paw formalin test. The characteristic curves showing the nociceptive responses to formalin injection in the paw, in control and FSS animals, treated with vehicle or morphine 5 mg/kg, are illustrated in Fig. 5D.

In the acute phase, statistical analyses showed significant effect for both morphine treatment ($F_{(1,49)}= 52.26$, $p < 0.0001$) and stress ($F_{(1,49)}= 9.153$ $p < 0.01$) factors, but not for treatment x stress interaction ($F_{(1,49)}= 0.095$ $p > 0.05$). In the inflammatory phase, only stress factor had a significant effect (treatment: $F_{(1,49)}= 2.997$, $p > 0.05$; stress: $F_{(1,49)}= 9.01$ $p < 0.01$; treatment x stress interaction: $F_{(1,49)}= 0.648$, $p > 0.05$).

FSS slightly increased the nociceptive response to formalin injection in the acute phase ($p < 0.05$), but not in the inflammatory one.

Morphine reduced the number of nociceptive behaviours in a similar manner in both stressed and control animals in the acute phase. In the inflammatory phase there was a statistically significant difference between both, control and stressed, groups treated with morphine.

Thus, FSS did not affect the antinociceptive effect of morphine in the acute phase. On the other hand, in the inflammatory phase although we did not see a statistically significant effect of morphine in the control group, it reduced by a 17.86% the total nociceptive behaviours in control animals, whilst only a 5.64% in the FSS group.

3.3. Involvement of opioid receptors in the reduced effect of morphine in stressed animals

The following experiments were performed to study if opioid receptors were involved in the mechanisms by which stress reduces the antinociceptive effect of morphine.

Because we observed a less robust effect in the tests performed in the limbs we decided to use an orofacial one.

Additionally, to reduce the number of animals (for ethical reasons) and because similar results were observed in the orofacial HS and formalin tests we tested the antagonist in the orofacial HS test.

The administration of naloxone prior to each session of forced swimming, recovered the antinociceptive effect of morphine; consequently, in the naloxone and morphine treated group a significant decrease in nociceptive responses, compared to FSS rats treated just with morphine was found ($p = 0.005$) (Fig. 6). Naloxone alone did not modify the response to HS ($p > 0.05$).

3.4. Effect of FSS and morphine on gastrointestinal function

When the effect of FSS and morphine in different GI regions was analysed, we observed that stress did not affect gastric emptying but slightly delayed small intestine emptying, with statistically significant differences at T6 ($p > 0.01$) (Fig. 7A and B). Similarly, when the effect of the morphine treatment was evaluated on the GI transit of the control and FSS groups, it was observed that neither the transit of the stomach nor small intestine were affected.

As shown in Fig. 7 C, stress alone did not modify the motility of the caecum, however, morphine (2.5 and 5 mg/kg) delayed the filling of the caecum in control and stressed animals at T2 (control MF2.5: $p < 0.01$; control MF5, FSS MF2.5, FSS MF5: $p < 0.001$), and at T4 in the control group with the dose of 5 mg/kg ($p < 0.05$).

As in the caecum, stress alone did not alter the motility in the colorectum either (Fig. 7D), although transit of the colorectum was significantly delayed in animals treated with both doses of morphine at T4 and T6 ($p < 0.001$).

Thus, morphine delayed GI transit in both caecum and colorectum and its effect was not affected by FSS.

4. Discussion

In the present research, the effects of stress on nociception and on the effect of morphine in two different anatomic areas: orofacial (trigeminal innervation) and limb (spinal innervation) and two different types of pain (nociceptive and inflammatory) were assessed in the same study, for the first time.

The main findings are that short-term stress reduces significantly the antinociceptive effect of morphine when the pain is orofacial, although it does not modify muscular or inflammatory pain in an important manner either in the orofacial area or in the limbs. On the other hand, this type of stress does not affect morphine-induced GI transit modification.

Before evaluating nociception, we have tried to rule out two possible behavioural problems: anxiety and locomotor alterations, to discard an important systemic effect of stress.

Some authors have detected anxiety induced by long lasting stress models [35], thus our first aim was to detect the presence of anxiety due to FSS. Therefore, we used the plus maze test because it is accepted to be quite specific to detect this alteration [36]. We did not find anxiety

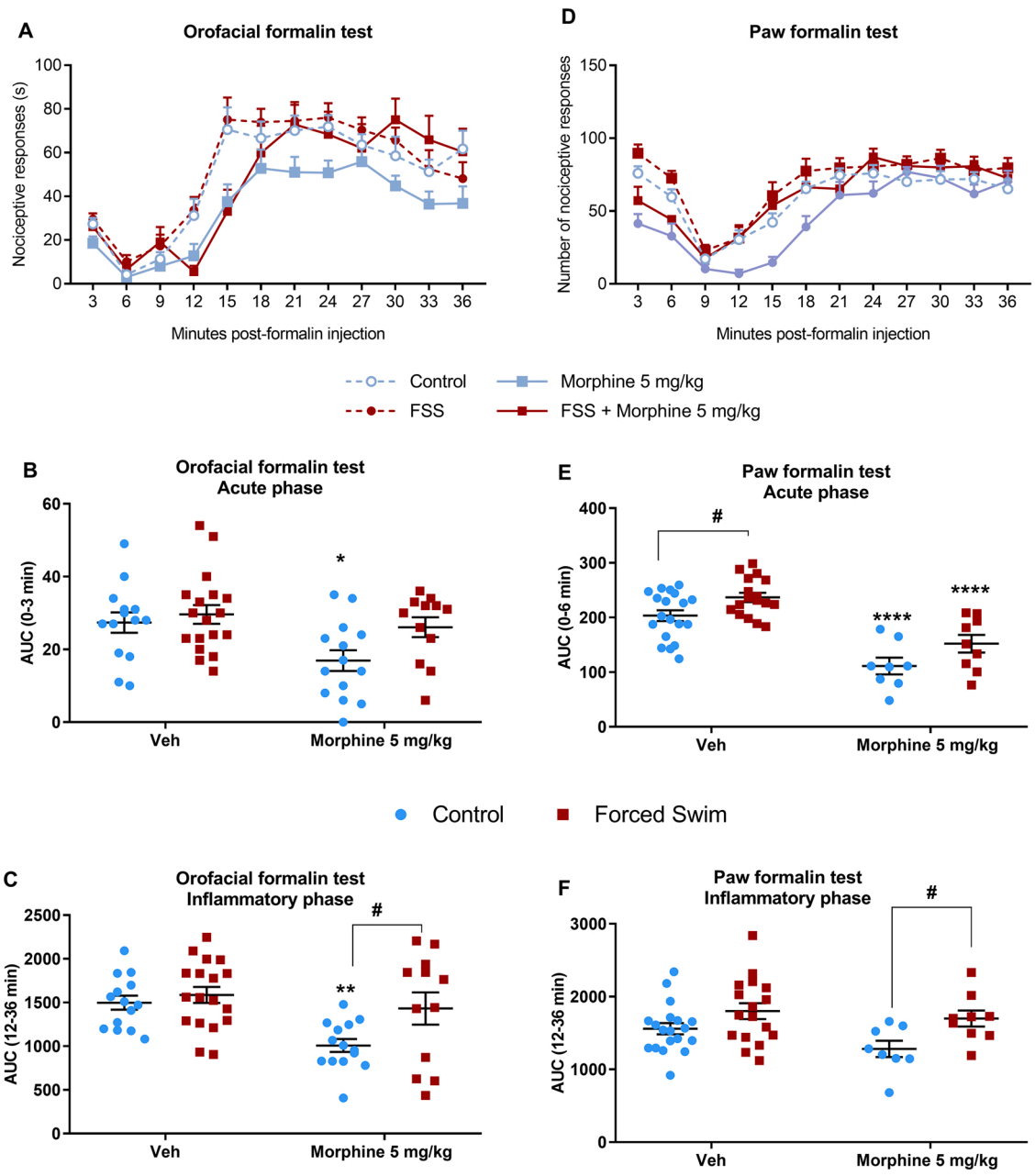

Fig. 5. Nociceptive behaviour induced by formalin in the vibrissae and paw. Panels A and D represent the time course of formalin-induced nociceptive behaviours in orofacial and paw regions, respectively, in control (non-stressed) and FSS rats, treated with vehicle and morphine 5 mg/kg. Each point expresses the mean ± SEM of nociceptive behaviours in seconds (A) or number (D) in three-min periods during 36 min. Panels B, C, E and F show the nociception induced by orofacial (B, C) and paw (E, F) formalin injection. Scatterplots represent the mean ± SEM of the area under curve (AUC) in the acute phase (B, E) and in the inflammatory phase (C, F) in control and FSS animals treated with vehicle (Veh) and morphine 5 mg/kg. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ *vs.* Veh; #$p < 0.05$ *vs.* non-stressed animals. Two-way ANOVA, Sidak's multiple comparisons *post hoc* test, $N \geq 10$.

behaviour in FSS animals.

In the actimeter test, our results show that FSS animals moved slightly less than control ones, which is in line with previous studies [37,38]. In addition, at the tested doses, morphine did not significantly alter the spontaneous locomotion of control animals and reversed the effect detected on animals under FSS. Furthermore, when nociception was assessed, no decrease in nociceptive responses was found in FSS animals, supporting the idea that modifications in spontaneous motility are not significant enough to alter the assessment of nociception. To date, the effects of stress and morphine on locomotor activity are not entirely understood, although it has been shown to be increased by the release of corticosteroids during the stress procedures [39]. Additionally, an upregulation of the mu opioid receptor in the tegmental ventral area and midbrain seems to be responsible, at least partly, of this effect [40,41]. Thus, both, stress and morphine have shown to induce similar effects potentiating excitatory and inhibitory synapses [42]. Possibly these mechanisms explain why, in our study, morphine increased the locomotor activity in the stressed rats.

Because the formalin test has been widely used to evaluate the effect of stress in previous studies [28,29,7], it was our choice to study inflammatory pain.

The effect of FSS on nociceptive behaviour to formalin injection in the paw has been previously studied and the results are controversial. While some authors have demonstrated hyperalgesia in both the acute and inflammatory phases [29] or only in the inflammatory phase [21,43], others have not observed any of these alterations [44]. It should be

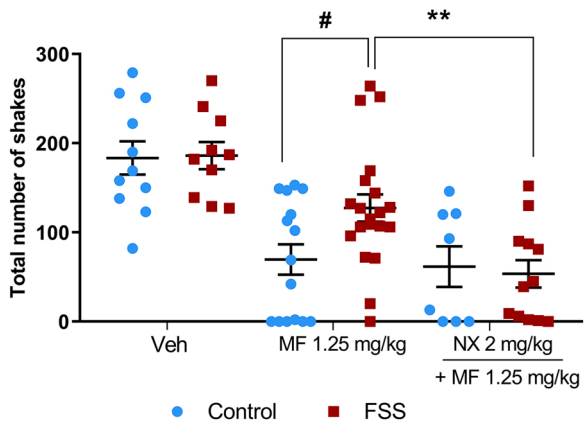

Fig. 6. Nociceptive responses to HS injection on masseter in control and FSS rats with different treatments. Scatterplots show the mean ± SEM of the total number of hind paw shakes in rats treated with morphine (MF) 1.25 mg/kg and pretreated with naloxone (NX) before each swimming session. * $p < 0.05$ *vs.* control intragroup; ** $p < 0.01$ *vs.* FSS-MF 1.25. One-way ANOVA, Sidak's multiple comparison *post hoc* test, $N \geq 10$.

noted that the methodologies and strains of rats are different across studies, which may justify the different results [44]. Under our conditions, repeated exposure to FSS overall did not modify orofacial inflammatory pain with only a slight increase in the nociceptive behaviours in the paw formalin test during the acute phase.

Although there are very few preclinical studies addressing the effect of stress on muscle pain, it has been reported that chronic or repeated stress is particularly effective in promoting or increasing muscle pain. For example, early-life stress or repeated cold stress produces muscular mechanical hyperalgesia [45,46]. Additionally, clinical and preclinical studies have related chronic stress to hyperalgesia or enhanced nociception in the masticatory muscles [47-49].

The level of stress used here did not modify nociception in the masseter or the gastrocnemius. These results are very similar to those obtained in the formalin test; it seems that the stressor used is not intense or long enough to modify nociception. This is in line with a previous study in which FSS for three days was defined as "short-term stress" as it did not induce significant changes in basal nociception [10].

Regarding the effects of morphine, a single dose was selected from a pilot study, to test its antinociceptive effect in the formalin test in stressed and control animals. At the tested dose, the effect of this opioid did not reach a statistically significant antinociceptive effect in the inflammatory phase in the paw, thus this finding corroborates our previous finding that opioids have a higher efficacy in the orofacial muscles than those innervated spinally [25]. On the other hand, and as expected, morphine reduced the nociceptive behaviour induced by HS in a dose-dependent manner in control animals in both the masseter and the gastrocnemius muscles [25].

When the effect of morphine was tested in the stressed animals, a decrease in its antinociceptive effect was found, which is in accordance with previous studies which have used longer stress protocols [50,51,8]. A study performed by Suarez-Roca et al., 2006 also found a reduced antinociceptive effect of morphine after three days of forced swimming in the hot plate test, at greater doses (7.5 mg/kg) but not at lower doses (3 mg/kg). In this study the authors did not evaluate the locomotor activity. Considering that both stress and morphine at high doses affect locomotor activity, and how both interact to finally affect locomotor activity is to date not sufficiently known, the differences found between both studies could be due to the doses of morphine and the tests which have been used.

Interestingly, under our experimental conditions, there was a reduction in the antinociceptive effect of morphine in the orofacial acute nociceptive tests (HS and first phase of formalin), whilst this did not happen in the limbs. Previously, we have found that peripherally acting opioids have an antinociceptive effect in orofacial pain but not in the limbs [19] and that there are more μ opioid receptors in the trigeminal than L5 dorsal root ganglia [15]. Therefore, the orofacial area seems to be more susceptible to peripheral opioid analgesia. Although the mechanisms underlying the difference in the loss of morphine effect between the two areas cannot be explained at this stage, our results could indicate that peripheral opioid receptors are more vulnerable to stress.

The reduction in the efficacy of morphine could be explained by the fact that different stressors cause a reduction in the μ opioid receptor binding capacity in rodents and humans, due to a decrease in μ opioid receptors, in different structures of the CNS implicated in pain transmission [52-54].

Authors hypothesize that exposure to stress triggers the release of endogenous opioid peptides causing a down-regulation of opioid receptors, as μ opioid receptor internalization can be induced by endogenous opioids [55-57]. Although several studies have studied the effects of stress on the endogenous opioid system, we antagonized for the first time the effects of stress with naloxone, thus our results confirm this hypothesis, because when animals were pretreated with naloxone before FSS, morphine conserved its antinociceptive effect. This is most probably due to the antagonism of naloxone on opioid receptors thus these receptors become protected against the binding of endogenous opioids and, consequently, against receptor internalization, and therefore morphine conserves its antinociceptive effect.

Finally, we aimed to study if stress could also affect one of the most common opioid induced adverse effects, *i.e.* constipation. This was performed through radiographic methods. In a previous radiographic study, 10 mg/kg of morphine reduced rat gastric emptying and intestinal motility, but its effects were much more pronounced in the intestinal regions [58]. In the present study, lower doses of morphine (2.5 and 5 mg/kg) still reduced intestinal transit but did not affect gastric emptying, suggesting that the intestines are more sensitive to morphine than the stomach.

Overall, we did not find any effect of stress on GI transit; previous studies have shown that GI motility tends to normalize when homotypic stress is applied [2]. Curiously, there was a slight delay in the transit of the small intestine, which is in line with our previous study, although the time of stress induction is different in both studies [59]. Nevertheless, FSS did not modify the effect of morphine on GI motility. This is comparable to results previously obtained using acute restraint stress [60], suggesting that opioid-induced GI dysmotility may be universally resistant to stress. Thus, the mechanisms by which stress modifies the antinociceptive effect of morphine, but not of constipation seem to be different, which should be ascertained with additional experiments.

To conclude, according to our experimental conditions, exposure to forced swimming stress for three days does not significantly alter the perception of inflammatory or muscular pain. Additionally, it does not induce anxiety or GI transit disorders. However, it does produce a marked decrease in the analgesic effect of morphine, mainly when the pain is located in the orofacial region. On the other hand, forced swimming stress does not reduce the constipation induced by morphine.

Conclusion

Short term stress may decrease opioid analgesia when pain is localized at the orofacial area although it does not modify nociception. Therefore, the prevention or coping with the daily stressors may be essential to obtain an adequate opiate analgesia in the management of pain.

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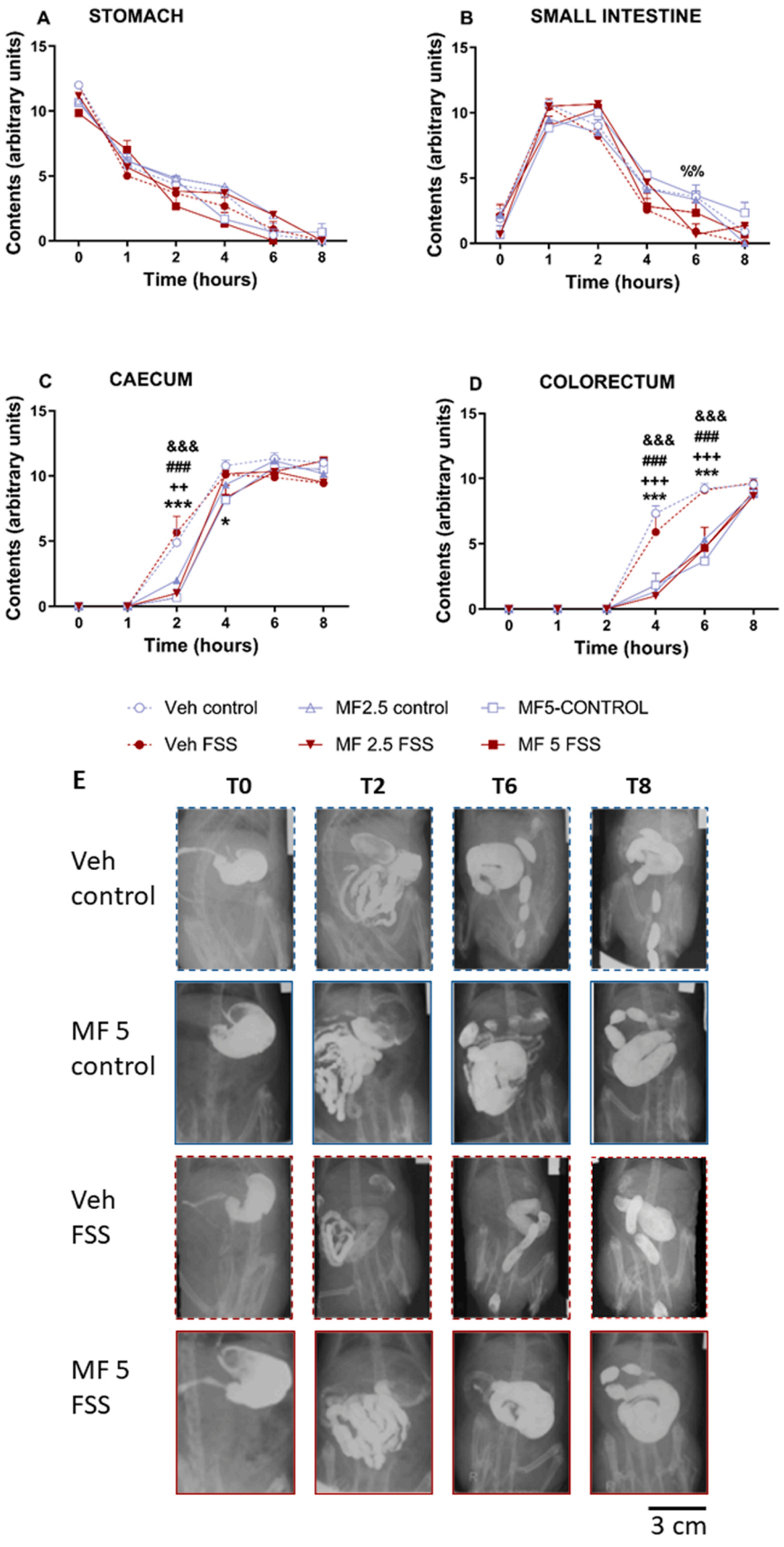

Fig. 7. Effect of morphine in control and FSS animals in the GI transit. Radiographic analysis of the effect of morphine (MF) (2.5 and 5 mg/kg) on GI motor function in control and stressed rats by forced swim stress (FSS). Curves show barium transit in the stomach (A), small intestine (B), caecum (C) and colorectum (D), using a semi-quantitative score. Panel E shows representative X-rays obtained from control and stressed rats treated with vehicle and MF 5 at 0, 2, 6 and 8 h after barium administration. Results are shown as mean ± SEM * $p < 0.05$, *** $p < 0.001$: MF 5 *vs.* control; + + $p < 0.01$, + ++ $p < 0.001$: MF 2.5 *vs.* control; ### $p < 0.001$: MF 2.5 FSS *vs.* FSS; &&&$p < 0.001$: MF 5 FSS *vs.* FSS; %%$p < 0.01$ FSS *vs.* control. Two-way ANOVA, Sidaḱs multiple comparisons *post hoc* test, $N \geq 6$.

CRediT authorship contribution statement

All authors listed above have sufficiently contributed to the project to be included as authors. A. Bagues, R. Girón and E.M. Sánchez-Robles have directly participated in the execution of the experimental behavioural work. M.I. Martín-Fontelles, A. Bagues, E.M. Sánchez-Robles an R. Abalo contributed to the conception, design of the study and wrote the manuscript. C. Goicoechea and R. Girón contributed to the elaboration of the manuscript. All the authors have reviewed the manuscript critically.

Declaration of Competing Interest

The authors state there are no conflicts of interest to be declare.

Data Availability

Data will be made available on request.

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References

[1] J.P. Herman, Neural control of chronic stress adaptation, Front. Behav. Neurosci. (2013), https://doi.org/10.3389/fnbeh.2013.00061.
[2] Y. Yang, R. Babygirija, J. Zheng, B. Shi, W. Sun, X. Zheng, F. Zhang, Y. Cao, Central neuropeptide y plays an important role in mediating the adaptation mechanism against chronic stress in male rats, Endocrinology 159 (2018) 1525–1536, https://doi.org/10.1210/en.2018-00045.
[3] P.W. Gold, The organization of the stress system and its dysregulation in depressive illness, Mol. Psychiatry 20 (2015) 32–47, https://doi.org/10.1038/mp.2014.163.
[4] R.K. Butler, D.P. Finn, Stress-induced analgesia, Prog. Neurobiol. 88 (2009) 184–202, https://doi.org/10.1016/J.PNEUROBIO.2009.04.003.
[5] E.M. Jennings, B.N. Okine, M. Roche, D.P. Finn, Stress-induced hyperalgesia, Prog. Neurobiol. 121 (2014) 1–18, https://doi.org/10.1016/J.PNEUROBIO.2014.06.003.
[6] I.V. Belozertseva, A.Y. Bespalov, Effects of NMDA receptor channel blockers, dizocilpine and memantine, on the development of opiate analgesic tolerance induced by repeated morphine exposures or social defeats in mice, Naunyn Schmiede Arch. Pharm. 358 (1998) 270–274, https://doi.org/10.1007/PL00005252.
[7] G.H. Gameiro, A. Da Silva Andrade, M. De Castro, L.F. Pereira, C.H. Tambeli, M. C. Ferraz De Arruda Veiga, The effects of restraint stress on nociceptive responses induced by formalin injected in rat's TMJ, Pharmacol. Biochem. Behav. 82 (2005) 338–344, https://doi.org/10.1016/j.pbb.2005.09.003.
[8] I.L. Da Silva Torres, S.N.S. Cucco, M. Bassani, M.S. Duarte, P.P. Silveira, A. P. Vasconcellos, A.S. Tabajara, G. Dantas, F.U. Fontella, C. Dalmaz, M.B.C. Ferreira, Long-lasting delayed hyperalgesia after chronic restraint stress in rats - effect of morphine administration, Neurosci. Res. 45 (2003) 277–283, https://doi.org/10.1016/S0168-0102(02)00232-8.
[9] J.R. Piazza, S.T. Charles, M.J. Sliwinski, J. Mogle, D.M. Almeida, Affective reactivity to daily stressors and long-term risk of reporting a chronic physical health condition, (n.d.). https://doi.org/10.1007/s12160–012-9423–0.
[10] J. Cao, P.K. Wang, V. Tiwari, L. Liang, B.M. Lutz, K.R. Shieh, W.D. Zang, A. G. Kaufman, A. Bekker, X.Q. Gao, Y.X. Tao, Short-term pre- and post-operative stress prolongs incision-induced pain hypersensitivity without changing basal pain perception, Mol. Pain. 11 (2015) 73, https://doi.org/10.1186/s12990-015-0077-3.
[11] A. Korszun, E. Papadopoulos, M. Demitrack, C. Engleberg, L. Crofford, The relationship between temporomandibular disorders and stress-associated syndromes, Oral. Surg., Oral. Med., Oral. Pathol., Oral. Radiol., Endod. 86 (1998) 416–420, https://doi.org/10.1016/S1079-2104(98)90366-3.
[12] D. Bansevicius, R.H. Westgaard, T. Stiles, EMG activity and pain development in fibromyalgia patients exposed to mental stress of long duration, Scand. J. Rheumatol. 30 (2001) 92–98, https://doi.org/10.1080/03009740151095367.
[13] F. Mendelek, I. Caby, P. Pelayo, R.B. Kheir, The application of a classification-tree model for predicting low back pain prevalence among hospital staff, Arch. Environ. Occup. Health 68 (2013) 135–144, https://doi.org/10.1080/19338244.2012.663010.
[14] E. Kalso, J.E. Edwards, R.A. Moore, H.J. McQuay, Opioids in chronic non-cancer pain: systematic review of efficacy and safety, Pain 112 (2004) 372–380, https://doi.org/10.1016/j.pain.2004.09.019.
[15] A. Bagues, M.I. Martín, A. Higuera-Matas, J. Esteban-Hernández, E. Ambrosio, E. María Sánchez-Robles, Mu-opioid receptors in ganglia, but not in muscle, mediate peripheral analgesia in rat muscle pain, Anesth. Analg. 126 (2018) 1369–1376, https://doi.org/10.1213/ANE.0000000000002717.
[16] E. Rodriguez, K. Sakurai, J. Xu, Y. Chen, K. Toda, S. Zhao, B.X. Han, D. Ryu, H. Yin, W. Liedtke, F. Wang, A craniofacial-specific monosynaptic circuit enables heightened affective pain, Nat. Neurosci. 20 (2017) 1734–1743, https://doi.org/10.1038/S41593-017-0012-1.
[17] B. Michot, S. Bourgoin, F. Viguier, M. Hamon, V. Kayser, Differential effects of calcitonin gene-related peptide receptor blockade by olcegepant on mechanical allodynia induced by ligation of the infraorbital nerve vs the sciatic nerve in the rat, Pain 153 (2012) 1939–1948, https://doi.org/10.1016/J.PAIN.2012.06.009.
[18] M.K. Chung, S. Wang, J. Yang, I. Alshanqiti, F. Wei, J.Y. Ro, Neural pathways of craniofacial muscle pain: implications for novel treatments, J. Dent. Res. 99 (2020) 1004–1012, https://doi.org/10.1177/0022034520919384.
[19] E.M. Sánchez, A. Bagües, M.I. Martín, Contributions of peripheral and central opioid receptors to antinociception in rat muscle pain models, Pharm. Biochem Behav. 96 (2010) 488–495, https://doi.org/10.1016/J.PBB.2010.07.009.
[20] M. Zimmermann, Ethical guidelines for investigations of experimental, Pain. Conscious Anim. Pain. 16 (1983) 109–110, https://doi.org/10.1016/0304-3959(83)90201-4.
[21] L. Quintero, R. Cardenas, H. Suarez-Roca, Stress-induced hyperalgesia is associated with a reduced and delayed GABA inhibitory control that enhances post-synaptic NMDA receptor activation in the spinal cord, Pain 152 (2011) 1909–1922, https://doi.org/10.1016/j.pain.2011.04.017.
[22] A.A. Walf, C.A. Frye, The use of the elevated plus maze as an assay of anxiety-related behavior in rodents, Nat. Protoc. 2 (2007) 322–328, https://doi.org/10.1038/NPROT.2007.44.
[23] A. Bagüés, M.I. Martín, E.M. Sánchez-Robles, Involvement of central and peripheral cannabinoid receptors on antinociceptive effect of tetrahydrocannabinol in muscle pain, Eur. J. Pharmacol. 745 (2014) 69–75, https://doi.org/10.1016/j.ejphar.2014.10.016.
[24] J.Y. Ro, N. Capra, R. Masri, Development of a behavioral assessment of craniofacial muscle pain in lightly anesthetized rats, Pain 104 (2003) 179–185, https://doi.org/10.1016/S0304-3959(03)00005-8.
[25] E.M. Sánchez, A. Bagües, M.I. Martín, Contributions of peripheral and central opioid receptors to antinociception in rat muscle pain models, Pharmacol. Biochem. Behav. 96 (2010) 488–495, https://doi.org/10.1016/J.PBB.2010.07.009.
[26] E.M.S. Robles, A.B. Arias, M.I.M. Fontelles, Cannabinoids and muscular pain. Effectiveness of the local administration in rat, Eur. J. Pain. (U. Kingd.) 16 (2012) 1116–1127, https://doi.org/10.1002/j.1532-2149.2012.00115.x.
[27] E. Burgos, D. Pascual, M.I. Martín, C. Goicoechea, Antinociceptive effect of the cannabinoid agonist, WIN 55,212-2, in the orofacial and temporomandibular formalin tests, Eur. J. Pain. 14 (2010) 40–48, https://doi.org/10.1016/j.ejpain.2009.02.003.
[28] L. Quintero, M. Moreno, C. Avila, J. Arcaya, W. Maixner, H. Suarez-Roca, Long-lasting delayed hyperalgesia after subchronic swim stress, Pharm. Biochem Behav. 67 (2000) 449–458, https://doi.org/10.1016/S0091-3057(00)00374-9.
[29] H. Imbe, K. Okamoto, T. Donishi, E. Senba, A. Kimura, Involvement of descending facilitation from the rostral ventromedial medulla in the enhancement of formalin-evoked nocifensive behavior following repeated forced swim stress, Brain Res. 1329 (2010) 103–112, https://doi.org/10.1016/J.BRAINRES.2010.03.006.
[30] M.M. Garcia, C. Goicoechea, M. Avellanal, S. Traseira, M.I. Martín, E.M. Sánchez-Robles, Comparison of the antinociceptive profiles of morphine and oxycodone in two models of inflammatory and osteoarthritic pain in rat, Eur. J. Pharmacol. 854 (2019) 109–118, https://doi.org/10.1016/j.ejphar.2019.04.011.
[31] A. Papilloud, V. Veenit, S. Tzanoulinou, O. Riccio, O. Zanoletti, I. Guillot de Suduiraut, J. Grosse, C. Sandi, Peripubertal stress-induced heightened aggression: modulation of the glucocorticoid receptor in the central amygdala and normalization by mifepristone treatment, Neuropsychopharmacology 44 (2019) 674–682, https://doi.org/10.1038/s41386-018-0110-0.
[32] P.A. Cabezos, G. Vera, M. Castillo, R. Fernández-Pujol, M.I. Martín, R. Abalo, Radiological study of gastrointestinal motor activity after acute cisplatin in the rat, Tempo Relatsh. Pica Auton. Neurosci.: Basic Clin. 141 (2008) 54–65, https://doi.org/10.1016/j.autneu.2008.05.004.
[33] R. Girón, I. Pérez-García, R. Abalo, X-ray analysis of gastrointestinal motility in conscious mice. Effects of morphine and comparison with rats. Neurogastroenterol. Motil. 28 (2016) 74–84, https://doi.org/10.1111/nmo.12699.
[34] H. Suarez-Roca, J.A. Silva, J.L. Arcaya, L. Quintero, W. Maixner, L. Piñerua-Shuhaibar, Role of μ-opioid and NMDA receptors in the development and maintenance of repeated swim stress-induced thermal hyperalgesia, Behav. Brain Res. 167 (2006) 205–211, https://doi.org/10.1016/j.bbr.2005.09.006.
[35] W. Lin, Y. Zhao, B. Cheng, H. Zhao, L. Miao, Q. Li, Y. Chen, M. Zhang, NMDAR and JNK activation in the spinal trigeminal nucleus caudalis contributes to masseter hyperalgesia induced by stress, Front. Cell. Neurosci. 13 (2019) 1–9, https://doi.org/10.3389/fncel.2019.00495.
[36] K. Belovicova, E. Bogi, K. Csatlosova, M. Dubovicky, Animal tests for anxiety-like and depression-like behavior in rats, Interdiscip. Toxicol. 10 (2017) 40–43, https://doi.org/10.1515/intox-2017-0006.
[37] A.C. Harris, C. Mattson, D. Shelley, M.G. Lesage, Restraint stress attenuates nicotine's locomotor stimulant but not discriminative stimulus effects in rats, Pharmacol. Biochem. Behav. 124 (2014) 92–100, https://doi.org/10.1016/j.pbb.2014.05.012.
[38] Y. Pan, W. Lin, W. Wang, X. Qi, D. Wang, M. Tang, The effects of central pro-and anti-inflammatory immune challenges on depressive-like behavior induced by chronic forced swim stress in rats, Behav. Brain Res. 247 (2013) 232–240, https://doi.org/10.1016/j.bbr.2013.03.031.
[39] T. Stöhr, O.F.X. Almeida, R. Landgraf, T.S. Shippenberg, F. Holsboer, R. Spanagel, Stress- and corticosteroid-induced modulation of the locomotor response to

morphine in rats, Behav. Brain Res. 103 (1999) 85–93, https://doi.org/10.1016/S0166-4328(99)00027-3.
[40] E.M. Nikulina, K.A. Miczek, R.P. Hammer, Prolonged effects of repeated social defeat stress on mRNA expression and function of μ-opioid receptors in the ventral tegmental area of rats, Neuropsychopharmacology 30 (2005) 1096–1103, https://doi.org/10.1038/sj.npp.1300658.
[41] M. Yamamoto, T. Komori, T. Matsumoto, K. Zhang, S. Miyahara, K. Shizuya, Y. Okazaki, Effects of single and repeated prolonged stress on mu-opioid receptor mRNA expression in rat gross hypothalamic and midbrain homogenates, Brain Res. 980 (2003) 191–196, https://doi.org/10.1016/S0006-8993(03)02969-X.
[42] A.M. Polter, K. Barcomb, R.W. Chen, P.M. Dingess, N.M. Graziane, T.E. Brown, J. A. Kauer, Constitutive activation of kappa opioid receptors at ventral tegmental area inhibitory synapses following acute stress, Elife 6 (2017) 1–21, https://doi.org/10.7554/eLife.23785.
[43] H. Suarez-Roca, L. Leal, J.A. Silva, L. Piñerua-Shuhaibar, L. Quintero, Reduced GABA neurotransmission underlies hyperalgesia induced by repeated forced swimming stress, Behav. Brain Res. 189 (2008) 159–169, https://doi.org/10.1016/j.bbr.2007.12.022.
[44] E.M. Jennings, B.N. Okine, W.M. Olango, M. Roche, D.P. Finn, Repeated forced swim stress differentially affects formalin-evoked nociceptive behaviour and the endocannabinoid system in stress normo-responsive and stress hyper-responsive rat strains, Prog. Neuro Psychopharmacol. Biol. Psychiatry 64 (2016) 181–189, https://doi.org/10.1016/j.pnpbp.2015.05.008.
[45] P.G. Green, X. Chen, P. Alvarez, L.F. Ferrari, J.D. Levine, Early-life stress produces muscle hyperalgesia and nociceptor sensitization in the adult rat, Pain 152 (2011) 2549–2556, https://doi.org/10.1016/j.pain.2011.07.021.
[46] T. Nasu, S. Murase, Y. Takeda-Uchimura, K. Mizumura, Intramuscularly injected neurotropin reduced muscular mechanical hyperalgesia induced by repeated cold stress in rats, Behav. Pharmacol. 29 (2018) 261–269, https://doi.org/10.1097/FBP.0000000000000313.
[47] K.H. Bendixen, A.J. Terkelsen, L. Baad-Hansen, B.E. Cairns, P. Svensson, Experimental stressors alter hypertonic saline-evoked masseter muscle pain and autonomic response, J. Orofac. Pain. 26 (2012) 191–205.
[48] F. Huang, M. Zhang, Y.-J. Chen, Q. Li, A.-Z. Wu, Psychological stress induces temporary masticatory muscle mechanical sensitivity in rats, J. Biomed. Biotechnol. 2011 (2011), 720603, https://doi.org/10.1155/2011/720603.
[49] Y.J. Zhao, Y. Liu, Q. Li, Y.H. Zhao, J. Wang, M. Zhang, Y.J. Chen, Involvement of trigeminal astrocyte activation in masseter hyperalgesia under stress, Physiol. Behav. 142 (2015) 57–65, https://doi.org/10.1016/j.physbeh.2015.02.005.
[50] G.H. Gameiro, P.H. Gameiro, A. da Silva Andrade, L.F. Pereira, M.T. Arthuri, F. K. Marcondes, M.C.F. de Arruda Veiga, Nociception- and anxiety-like behavior in rats submitted to different periods of restraint stress, Physiol. Behav. 87 (2006) 643–649, https://doi.org/10.1016/j.physbeh.2005.12.007.
[51] H. Neyama, N. Dozono, H. Ueda, NR2A-NMDA receptor blockade reverses the lack of morphine analgesia without affecting chronic pain status in a fibromyalgia-like mouse model, J. Pharmacol. Exp. Ther. 373 (2020) 103–122, https://doi.org/10.1124/jpet.119.262642.
[52] S.A. Laredo, M.Q. Steinman, C.F. Robles, E. Ferrer, B.J. Ragen, B.C. Trainor, Effects of defeat stress on behavioral flexibility in males and females: modulation by the mu-opioid receptor, Eur. J. Neurosci. 41 (2015) 434–441, https://doi.org/10.1111/EJN.12824.
[53] I. Liberzon, S.F. Taylor, K.L. Phan, J.C. Britton, L.M. Fig, J.A. Bueller, R.A. Koeppe, J.K. Zubieta, Altered central μ-Opioid receptor binding after psychological trauma, Biol. Psychiatry 61 (2007) 1030–1038, https://doi.org/10.1016/j.biopsych.2006.06.021.
[54] G. Dantas, I.L. Da Silva Torres, L.M. Crema, D.R. Lara, C. Dalmaz, Repeated restraint stress reduces opioid receptor binding in different rat CNS structures, Neurochem. Res. 30 (2005) 1–7, https://doi.org/10.1007/s11064-004-9679-2.
[55] C. Sternini, M. Spann, B. Anton, D.E. Keith, N.W. Bunnett, M. Von Zastrow, C. Evans, N.C. Brecha, Agonist-selective endocytosis of mu opioid receptor by neurons in vivo, Proc. Natl. Acad. Sci. USA 93 (1996) 9241, https://doi.org/10.1073/PNAS.93.17.9241.
[56] J.G. Minnis, S. Patierno, S.E. Kohlmeier, N.C. Brecha, M. Tonini, C. Sternini, Ligand-induced mu opioid receptor endocytosis and recycling in enteric neurons, Neuroscience 119 (2003) 33–42, https://doi.org/10.1016/S0306-4522(03)00135-0.
[57] A.A. Hawranko, D.J. Smith, Stress reduces morphine's antinociceptive potency: dependence upon spinal cholecystokinin processes, Brain Res. (1999), https://doi.org/10.1016/S0006-8993(99)01216-0.
[58] R. Girón, I. Pérez-García, R. Abalo, X-ray analysis of gastrointestinal motility in conscious mice. Effects of morphine and comparison with rats. Neurogastroenterol. Motil. 28 (2016) 74–84, https://doi.org/10.1111/nmo.12699.
[59] A. Bagues, Y. Lopez-Tofiño, C. Galvez-Robleño, R. Abalo, Effects of two different acute and subchronic stressors on gastrointestinal transit in the rat: A radiographic analysis, Neurogastroenterol. Motil. 33 (2021), https://doi.org/10.1111/NMO.14232.
[60] B.D. Appelbaum, S.G. Holtzman, Restraint stress has no effect on morphine-induced inhibition of gastrointestinal transit in the rat, Physiol. Behav. 34 (1985) 995–997, https://doi.org/10.1016/0031-9384(85)90026-5.