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Sex differences in the impact of nerve injury on locus coeruleus function and behaviour in mice

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Abstract

Chronic pain often coexists with anxiety and depression, particularly in women. The locus coeruleus (LC), a noradrenergic nucleus, modulates pain and emotional states and shows sex-specific structural and functional properties in preclinical studies. Moreover, clinical evidence indicates sex differences in the experience of chronic pain and its emotional impact. These findings highlight the need to explore the LC mechanisms underlying sex-dependent differences in chronic pain. In this study, we examined the sex-specific role of the LC in chronic pain and its emotional and cognitive consequences. Male and female mice exposed to the chronic constriction injury (CCI) model (2/3 and 7/11 weeks postinjury) were evaluated for sensory responses (von Frey, acetone, plantar tests), anxiety-like behavior (open field), depressive-like behavior (tail suspension test, forced swimming test), and cognitive performance (novel object recognition test). We also analyzed the number, somatodendritic volume, and electrophysiological properties of noradrenergic LC neurons. Finally, we assessed the effects of chemogenetic LC inhibition on anxiety- and depressive-like behavior, as well as fear responses. Nerve injury induced immediate sensory hypersensitivity in both sexes. Depressive-like behavior and cognitive deficits appeared only after prolonged injury. Notably, anxiety-like behavior and enhanced fear conditioning were exclusive to CCI male mice and correlated with LC-specific changes: increased cell number and somatodendritic volume, as well as heightened excitability. In female mice, however, neuronal excitability was reduced. Chemogenetic LC inhibition reversed anxiety- and depressive-like behaviors and fear responses only in CCI male mice. These findings show that neuropathic pain elicits sex-specific emotional and neural responses in the LC, highlighting the need for sex-specific approaches when investigating LC function and developing targeted interventions.

Keywords: Chronic pain, Sex differences, Locus coeruleus, Anxiety, Depression, Fear conditioning

1. Introduction

Chronic pain affects 20% to 30% of adults globally, and approximately 40% of those individuals also suffer from depression and anxiety. In most studies, women have a significantly higher prevalence of chronic pain and are more likely to exhibit comorbid depression and anxiety.[1] Despite this, most preclinical studies on chronic pain comorbidities have focused on male rodents, thereby reflecting primarily male biological mechanisms. This scenario underscores the need to include both male and female rodent models in chronic pain research to better understand sex differences and improve the relevance of findings.

Some studies, mainly in rodents, have reported sexual dimorphism in several brain structures, including the locus coeruleus (LC).[21] The LC, a noradrenergic nucleus located in the brainstem, integrates signals related to attention, anxiety, stress response, arousal/sleep, learning and memory, sensory processing, pain, and reward processing.[14] In naïve rodents, the LC in female rats receives more stress-related afferents from regions such as the central amygdala and the bed nucleus of the stria terminalis.[42] Furthermore, female rats exhibit a more complex dendritic morphology in the LC compared with male rats.[5,28] Accordingly, our previous studies have identified pronounced sex differences in the LC structure and LC-related behaviours in naïve mice.[30] Although most of this evidence derives from animal models, there is also some evidence of structural sex differences in the human LC. In this regard, previous studies have estimated the number of LC neurons to be approximately 15,700 in men and 18,300 in women, suggesting a possible anatomical sex difference in the human LC.[10,36]

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

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Importantly, the LC also appears to contribute to the development of affective and cognitive alterations associated with chronic neuropathic pain. In male rodents experiencing chronic neuropathic pain, caused by disease or damage to the somatosensory system, evidence suggests that, as time passes after injury, increased LC activity leads to heightened anxiety, aversive learning and memory, and behavioural despair.[11,25] However, the potential influence of sex on these LC-related alterations remains poorly understood. Studying these processes in a sex-specific manner may help elucidate the neurobiological mechanisms underlying sex differences in the emotional and cognitive consequences of chronic pain.

Therefore, this study investigates sex differences in the LC of neuropathic pain mice, focusing on its role in modulating sensory, emotional, and cognitive outcomes.

2. Materials and methods

2.1. Animals and experimental design

Experiments were conducted on adult (8-12 weeks old) male and female wild-type or tyrosine hydroxylase-Cre (TH:Cre) transgenic C57BL/6J mice (founders provided by EMMA INFRAFRONTIER, EM:00254).[20] Mice were housed in groups of 3 to 5 per cage, in separate rooms according to their sex under standard laboratory conditions (22°C, 12-hour light-dark cycle; lights on at 7:00 AM) with ad libitum access to food and water. All procedures were approved by the Committee for Animal Experimentation of the University of Cádiz and the UPV/EHU (M20/2021/234 and M30/2021/235), in compliance with the European Commission's Directive (2010/63/EU), Spanish Law (RD 53/2013), and ARRIVE guidelines.

Experimental time points were set at 2 to 3 weeks (chronic constriction injury [CCI]-2/3w) or 7 to 11 weeks (CCI-7/11w) post-nerve injury. Mice were randomly assigned to experimental cohorts (#1 to #12) subjected to different behavioural paradigms (see Supplementary Table 1, http://links.lww.com/PAIN/C454), and no additional strategies were employed to minimize potential confounders. All animals were habituated to the testing room for 30 minutes before the experiments. All behavioural approaches were conducted in the light phase (starting from 8 AM) under 13 lux lighting, and the apparatuses were cleaned with 70% ethanol between animals, especially between sexes. Experiments and analyses were performed blind to the assigned group or treatment.

2.2. Neuropathic pain model

Chronic constriction injury of the sciatic nerve was used as a model for neuropathic pain (**Fig. 1A**).[18] Mice were anaesthetised on 4% isoflurane and maintained on 1.5% to 2.5% isoflurane in a mixture of O_2 at a flow rate of 0.5 L/min. The left hind paw was shaved at the mid-thigh level, and after a skin incision, the gluteus superficialis and the biceps femoris muscles were separated using curved forceps. The left sciatic nerve was exposed proximal to the trifurcation, and 3 chromic gut (6-0) ligatures were loosely tied around the nerve at 1-mm intervals. The skin layers were subsequently closed using 5-0 silk thread. Sham-operated mice underwent the same procedure, except for nerve ligation.

2.3. Designer receptors exclusively activated by designer drugs virus injection

Male and female TH:Cre sham and CCI-7/11w mice were bilaterally injected into the LC with a Cre-dependent Designer Receptors Exclusively Activated by Designer Drugs (DREADD) inhibitor virus (AAV2/hSyn-DIO-hM4D[Gi]-mCherry, hM4D[Gi]-DREADD, from Virus Vector Core, Gene Therapy Center Vector Core at the University of North Carolina).[27] In brief, mice were anaesthetised and placed in a stereotaxic frame (Kopf Instruments, Tujunga). Bupivacaine (2 mg/kg) and lidocaine (3.5 mg/kg) were administered subcutaneously in the incision area before surgery. The DREADD virus (titer 3×10^{12} vg/mL) was bilaterally injected at a volume of 0.5 μL into the LC (Anterior-Posterior, AP: −5.5 mm, Medial-Lateral, ML: ±1 mm, Dorsal-Ventral, DV: 3 mm),[37] and the skin was closed using 5-0 silk thread. DREADD expression in the LC was verified at the end of the experiments in the LC and other noradrenergic areas, such as A5 and A7 (Fig. S4A, http://links.lww.com/PAIN/C454). Behavioural testing was carried out 3 weeks after virus injections to maximize vector expression, coinciding with the CCI-7/11w time point. Experimental groups included CCI-7/11w male and female mice receiving DREADD virus injections into the LC, which were administered intraperitoneally with either saline or clozapine-N-oxide (CNO, 3 mg/kg) for chemogenetic LC inhibition, 30 minutes before each behavioural test, with experimenters blind to the treatment. To control for any effects of the ligand, a group of male and female wild-type mice received injections of CNO (3 mg/kg, i.p.) and were subjected to behavioural tests.

2.4. Sensory, emotional, and cognitive assessment

2.4.1. Von Frey test

Mechanical hypersensitivity was assessed at CCI-2/3w and CCI-7/11w after injury using a Dynamic Plantar Aesthesiometer (Ugo Basile, Italy). After 30 minutes of habituation on a wire-mesh floor, a force ranging from 0 to 5 g over 10 seconds was applied to the plantar surface of the hind paws. The withdrawal threshold (grams) was determined as the average of 2 measurements that elicited a rapid paw withdrawal.[18]

2.4.2. Acetone test

The acetone evaporation test was used to assess cold hypersensitivity. Mice underwent a 30-minute habituation period in individual plastic cages placed on an elevated mesh floor. A 50-μL drop of acetone was applied to the plantar surface of each hind paw to induce evaporative cooling. The duration spent lifting, licking, and/or shaking of the hindlimb was monitored for 60 seconds, and the duration of response was calculated by averaging the results of 3 acetone applications.[13,15]

2.4.3. Plantar test

Thermal hyperalgesia was evaluated by the Hargreaves method.[19] Mice were habituated for 45 minutes in individual plastic cages on an elevated glass surface, and a radiant heat source (Plantar test device, Ugo Basile, Italy) was applied to the plantar surface of each hind paw at a constant intensity, with a 30-second cut-off to prevent skin damage. Two measurements were made, and the mean latency (in seconds) of the paw withdrawal was considered the thermal nociceptive threshold.[2]

2.4.4. Tail suspension test

Mice were suspended by the distal end of their tails and raised 20 cm above the floor.[8] The latency to the first episode of immobility and the total duration of immobility, defined as the

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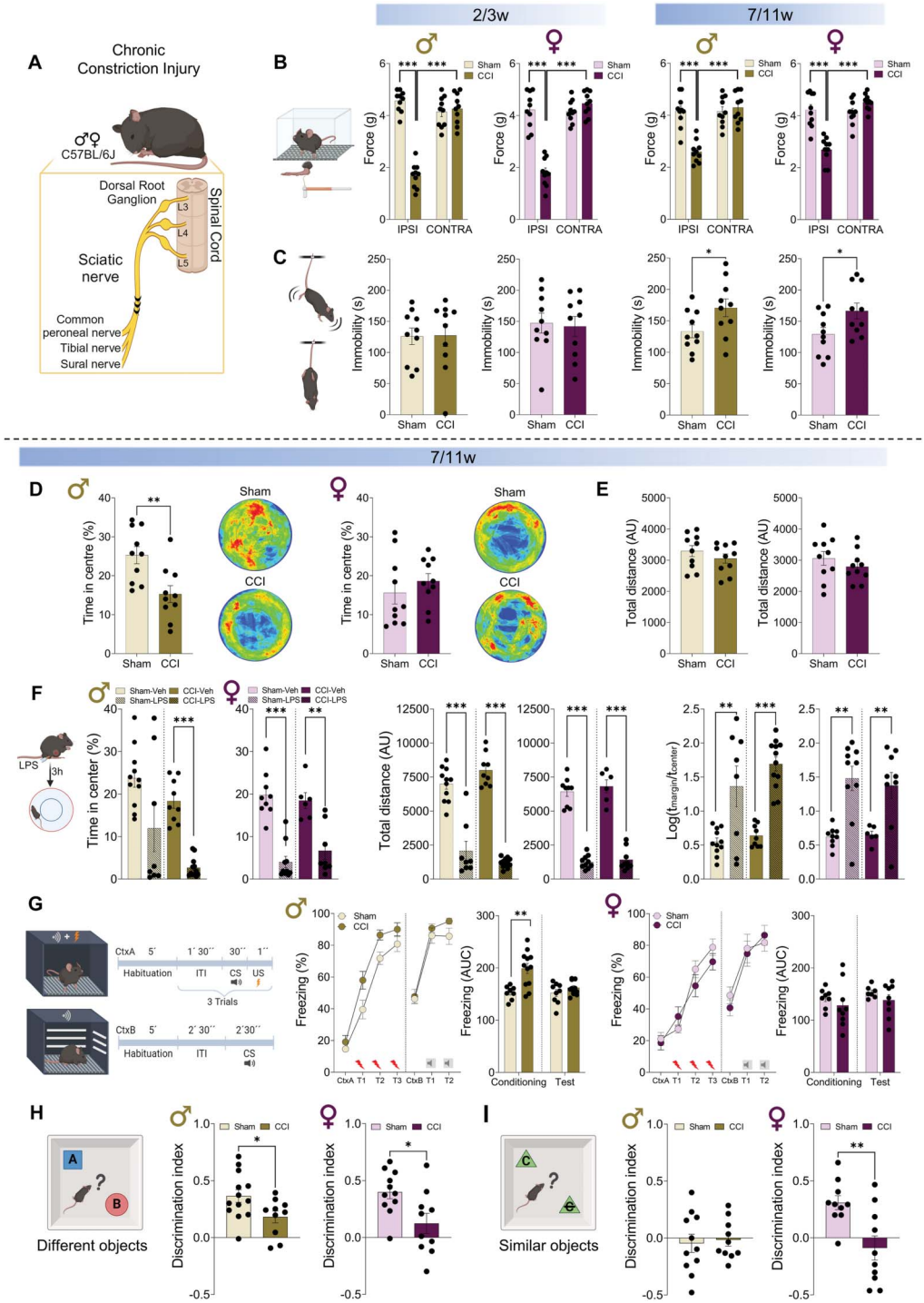

Figure 1. Sensory, emotional, and cognitive consequences of neuropathic pain in male and female C57BL/6J mice. (A) Schematic representation of the chronic constriction injury (CCI) surgery for subsequent behavioural experiments in male and female C57BL/6J mice. (B) Mechanical hypersensitivity measured using von Frey filament stimulation (0-5 g, in 10 seconds) in male and female mice, tested 2 to 3 weeks or 7 to 11 weeks post-surgery. (C) Depressive-like behaviour assessed by the tail suspension test (TST), represented by immobility time (in seconds) for both male and female mice over the testing period. (D) Impact of neuropathic pain (CCI-7/11w) on anxiety, evaluated using the open field (OF) test and represented as the percentage of time spent in the central zone in both male and female mice, along with representative heatmaps of activity and (E) locomotor activity quantified as the total distance travelled (in Arbitrary Units [AU]). (F) Stress-induced anxiety through acute lipopolysaccharide (LPS) systemic administration in the OF test, represented as the percentage of time spent in the central zone, total distance travelled, and thigmotaxis, expressed as Log (time in margin/time in centre) for male and female mice. (G) Response to an aversive stimulus in the fear conditioning (FC) test. Schematic representation of the FC procedure for the different phases: day 1 (Context A, Conditioning) and day 2 (Context B, Test). The percentage of freezing represented during the conditioned stimulus (CS) across trials and the area under the curve (AUC) of the percentage of freezing over time. (H) Schematic representations of the novel object recognition (NOR) experimental design to assess short-term memory (STM, learning index). Graph representing the discrimination index (DI) between objects following the STM protocols using a novel object (represented as B) that differed drastically from the familiar object (represented as A), and (I) using a novel object (represented as C crossed out) which presented strong similarity with the familiar one (represented as C) in both male and female mice. Data are presented as mean ± SEM of n = 7 to 13 mice per group: ***$P < 0.001$ vs contralateral paw and sham counterparts, 2-way ANOVA followed by Tukey post hoc test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs sham or vehicle treatment, Student *t* test. CONTRA, contralateral paw; IPSI, ipsilateral paw; ITI, intertrial interval; US, unconditioned stimulus.

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state of hanging by the tail without any active movements, were analysed during the 6-minute test session at CCI-2/3w and CCI-7/11w after injury. Additional behaviours were also assessed: (1) climbing—the animal climbs on its tail; (2) swinging—the animal moves its body from side to side; (3) curling—the animal performs twisting movements with its body actively; (4) clasping—the animal retracts its hind limbs towards the abdomen.

2.4.5. Forced swimming test

The forced swimming test (FST) was used to assess depressive-like behaviour.[9,38] Each animal was placed in a glass cylinder (10 cm diameter × 18 cm height) filled with water to a depth of 10 cm, at a temperature of 22 ± 1°C, for a 6-minute test. The sessions were recorded, and the latency to the first bout of immobility and the time spent immobile during the last 4 minutes of the testing period were analysed. Immobility behaviour was defined as the minimal movements necessary to keep the animal's head above water.

2.4.6. Open field test

Mice were individually placed in the centre of a white circular enclosure (40-cm diameter and 45-cm high walls) with 13 lux at the center and no bedding material was added. Mice were allowed to move freely for 10 minutes while being recorded. The centre of the arena was defined as an area comprising 50% of the total arena. The percentage of time spent in the centre (as an indicator of anxiety-like behaviour) and the total distance travelled (indicating locomotor activity) were analysed using the SMART video-tracking software (Panlab, S.L., Barcelona, Spain) at CCI-7/11w after injury. Thigmotaxis was calculated as the Log (time in margin/time in centre).[44]

2.4.7. Lipopolysaccharide administration

As lipopolysaccharide (LPS) rapidly induces sickness behaviour accompanied by anxiety-like responses, LPS (*Escherichia Coli* 0111:B4, Sigma-Aldrich, Madrid, Spain) in sterile phosphate buffer saline (PBS) was administered intraperitoneally at a single dose of 100 μg/kg.[35] Lipopolysaccharide-induced anxiety was assessed 3 hours postinjection using the open field (OF) test at CCI-7/11w, as previously described.

2.4.8. Fear conditioning test

In the fear conditioning test, a tone (30 seconds, 5 kHz, 50 dB) was used as a conditioned stimulus (CS), and a foot shock (1 second, 0.1 mA) was used as an unconditioned stimulus (US). Freezing was evaluated during the conditioned stimuli, as the absence of all movement except that necessary for respiration.[16] CCI-7/11w animals were fear conditioned (day 1) and context tested (day 2) in a Shuttle Box using 2 context options: Context A (CtxA) involves a black chamber with white light and scented with a 75% ethanol solution; Context B (CtxB) involves a black/white chamber with red light and scented with soapy water. Before conditioning and context testing, mice were habituated to the apparatus for 5 minutes. On the day of conditioning, mice were given 3 CS-US pairings during the CtxA. The intertrial interval was 90 seconds. The conditioned mice were tested for fear responses (freezing) 24 hours after conditioning. For this test, mice were assessed in CtxB and exposed to 1 CS of 150 seconds. The percentage of freezing was calculated manually by using SMART software and represented during each CS.

2.4.9. Novel object recognition test

The novel object recognition (NOR) paradigm was employed to assess short-term memory as a learning index, using 2 distinct approaches at CCI-7/11w. First, a classical NOR protocol with a new object completely different in shape, colour, and texture from the familiar one (Fig. S2C, http://links.lww.com/PAIN/C454). The second approach aimed to evaluate the ability to discern subtle differences between objects (Fig. S2H, http://links.lww.com/PAIN/C454).[30] The test was conducted in a 45 cm × 45 cm squared enclosure. Mice underwent a 10-minute habituation phase in the absence of objects, followed by a 10-minute training phase where they were allowed to explore 2 identical objects placed in opposite corners of the arena. To evaluate short-term memory, mice were returned to their home cages for a 1-hour delay period. Subsequently, they were reintroduced to the arena for a 10-minute test phase, with one of the familiar objects replaced by a novel object. The analysis measured preference for identical objects (%), total number of interactions with objects, latency to the novel object, and the discrimination index (DI), calculated as (Tnovel—Tfamiliar)/(Tnovel + Tfamiliar), where T represents the time exploring each object.[40] Animals showing a greater preference for 1 of the 2 familiar objects during the training phase were excluded from the analysis.

2.5. Immunohistochemistry and immunofluorescence

2.5.1. Perfusion and sample extraction

Mice were anaesthetised with 25% sodium pentobarbital and transcardially perfused with 0.9% saline solution followed by 4% paraformaldehyde (PFA) prepared in 0.1 M PBS. Brains were post-fixed in 4% PFA for 2 hours, then immersed in a 30% sucrose solution with 0.1% sodium azide in 0.1M phosphate buffer, and stored at 4°C for at least 1 overnight. Coronal sections (40 μm) were cut using a freezing microtome and preserved in cryoprotectant at 4°C until further analysis.

2.5.2. Structural and morphological studies

Free-floating immunohistochemistry was performed as previously described,[30] using 1 of 4 series of 40 μm-thick LC sections from 5 mice per group at CCI-2/3w and CCI-7/11w. First, endogenous peroxidase activity was blocked by incubating the sections with 0.3% H_2O_2 in PBS for 30 minutes, followed by a 1-hour incubation with 2.5% bovine serum albumin (BSA) in 0.1 M PBS containing 0.3% Triton X-100. Then, sections were incubated with primary anti-Tyrosine Hydroxylase antibodies (rabbit anti-TH, 1:1000; OPA1-04050, Millipore, Madrid, Spain) for 2 nights at 4°C. After washing, the sections were incubated with biotinylated donkey anti-rabbit antibodies (1:200, Jackson ImmunoResearch, Europe, United Kingdom) for 60 minutes at room temperature. Subsequently, the ultra-sensitive ABC peroxidase staining kit (1:1000, Thermo Scientific, Madrid, Spain) and DAB (3,3′-diaminobenzidine tetrahydrochloride) were used to visualise immunostaining. Sections were mounted on glass slides, cleared in xylene, and coverslipped using Dibutylphthalate Polystyrene Xylene (DPX) mounting medium. An Olympus BX60 microscope equipped with an Olympus DP74 camera was used to capture images with the same exposure and illumination settings for all groups. The total number of TH-positive cells in the LC was manually counted using the Cell Counter plugin in ImageJ (National Institutes of Health, Bethesda, MD), and the area occupied by TH-positive cells within the LC was measured in mm^2 using the wand tool. The density of TH+ neurons in the LC

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was determined by counting neurons and normalizing to the LC sampled area, expressed as neurons/mm^2.

Immunofluorescence analysis was conducted on all 40 μm-thick LC sections from a different set of 5 mice per group at CCI-2/3w and CCI-7/11w. The same primary antibody was used, followed by appropriate fluorophore-conjugated secondary antibodies (Donkey anti-rabbit Alexa 488, 1:1000; A-21206, Invitrogen, Madrid, Spain). After mounting on glass slides with a hard-setting antifade medium (Dako, Agilent, Madrid, Spain, S3023), the fluorescent signal was observed and captured using a 20X oil immersion objective on a confocal microscope (Olympus, Barcelona, Spain, FV1000) with consistent exposure settings. Imaging systematically covered the entire rostrocaudal extent of the LC (approximately 5.32 mm to 5.82 mm from Bregma). The LC volume was estimated based on the Cavalieri principle[32] following the formula $V = \Sigma A \times T$, where ΣA is the sum of the measured areas in all LC sections, and T is the distance between sections. Thus, the area occupied by the somas of the LC cells, as well as the somato-dendritic area, comprising the entire LC, was calculated in each 40 μm-spaced parallel LC section. These areas were automatically delineated using the wand tool in an 8-connected configuration mode to detect connected regions. Subsequently, the dendritic area was estimated by subtracting the soma area from the entire LC area. The representation of each area across all the LC sections was depicted, and the area under the curve was also calculated along the rostro-caudal axis. The results were expressed in mm^2 for areas and mm^3 for volumes.

2.5.3. Designer receptors exclusively activated by designer drugs expression in locus coeruleus-noradrenergic neurons

To evaluate the selective expression of DREADD in the LC and its absence in the A5 and A7 noradrenergic nuclei (Fig. S4A, http://links.lww.com/PAIN/C454), cryosections were incubated with a primary antibody cocktail to detect red fluorescent proteins (rat anti-RFP [mCherry], 1:500, 5F8, ChromoTek, Planegg, Germany) and dopamine β-hydroxylase (rabbit anti-DβH, 1:500, ab20948, Abcam, Cambridge, United Kingdom), followed by appropriate fluorophore-conjugated secondary antibodies (Streptavidin Alexa Fluor 568 conjugate, 1:1000, S11226, Invitrogen, Madrid, Spain; Donkey anti-rabbit Alexa 488, 1:1000, A-21206, Invitrogen, Madrid, Spain). Images were captured and analysed for colocalization using a Zeiss LSM 900 Airyscan 2 confocal microscope, and the percentage of the mCherry+ in DBH+ neurons was quantified in 4 randomly selected animals. In addition, animals lacking viral expression by misplaced LC injections were excluded from the analysis (n = 5-7), as they did not display the expected DREADD-induced phenotype (Fig. S4B, http://links.lww.com/PAIN/C454).

2.6. Western blot assay

The protein expression level of receptors that modulate the LC-noradrenergic system,[45] including cannabinoid receptor 1 (CB1) and corticotropin-releasing hormone receptor 1 (CRHR1), was evaluated in the LC of CCI-7/11w mice by western blotting. Locus coeruleus samples from 5 mice per group were dissected and pooled for protein isolation. Tissue homogenization was carried out through sonication in 200 μL of PBS (pH = 7) mixed with a protease inhibitor cocktail (Complete, Roche, Mannheim, Germany, 04693116001), followed by centrifugation at 12,000 rpm for 10 minutes at 4°C. Supernatants were collected and quantified using the Bradford method and then mixed with Laemmli sample buffer (BioRad, Madrid. Spain, 1610737). Furthermore, 20 μg/μL were loaded and separated in 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (110 V). Subsequently, 0.2 μm nitrocellulose membranes (BioRad, Madrid, Spain, 170459) were blocked in Tris-buffered saline supplemented with 0.1% Tween 20 and 5% BSA and then incubated with the following antibodies overnight: CB1 (Invitrogen, Madrid, Spain, PA1-743, 1/250) and CRHR1 (Invitrogen, Madrid, Spain, 720290, 1/1000). The next day, primary antibodies were washed, and membranes were incubated with their respective horseradish peroxidase-conjugated secondary antibodies for 1 hour and 30 minutes at room temperature and revealed using an ECL kit (Amersham Ibérica, Madrid, Spain, 12644055). Blots were imaged using the ChemiDoc Imaging System (BioRad, Madrid, Spain, 12003153) and quantified by densitometry with the NIH ImageJ software. Every densitometry is expressed as arbitrary units of optical density. β-actin (Sigma-Aldrich, Madrid, Spain A5441, 1/8000) was used as a loading control.[22,29]

2.7. Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings of LC neurons were performed at CCI-7w as previously described.[30] Sham and CCI-7w mice (5-6 male mice and 6 female mice per group) were sacrificed by decapitation under deep anesthesia (4% isoflurane), brains were removed and transferred to ice-cold artificial cerebrospinal fluid (ACSF, pH 7.4) equilibrated with 95% O_2 and 5% CO_2, and containing (in mM): 250 sucrose, 26 $NaHCO_3$, 1.25 $NaH_2PO_4.H_2O$, 0.5 $CaCl_2.2H_2O$, 10 $MgSO_4.7H_2O$, 10 D-glucose. Coronal sections of the brain containing the LC (220 μm thick) were obtained with a vibratome (VT1200S; Leica Microsystems, Wetzlar, Germany), and slices were incubated in warmed (30-35°C) ACSF for at least 30 minutes before recording. Each slice was transferred to a recording chamber that was perfused continuously with oxygenated ACSF at 32 to 34°C following our standard protocol.[33,39] Locus coeruleus neurons were visualized using infrared gradient contrast video microscopy (Eclipse workstation, Nikon, Madrid, Spain) and with a 60X water-immersion objective (Fluor 60X/1.00 W, Nikon, Madrid, Spain). The LC was identified as a dense and compact group of cells at the lateral border of the central gray and the fourth ventricle, just anterior to the genu of the facial nucleus. Recordings from individual LC neurons were obtained with pipettes (impedance, 3-6 MΩ) prepared from borosilicate glass capillaries (G150-4: Warner Instruments, Hamden, CR). The patch pipette was filled with a KGluconate-based solution containing (in mM): 130 KGluconate, 5 NaCl, 1 $MgCl_2.6H_2O$, 10 HEPES, 1 Na4EGTA, 2 MgATP, 0.5 NaGTP, and 10 Na_2PCr. The junction potential between the electrode solution and the external media (empirically estimated as 13 mV) was not corrected, and electrode signals were low-pass filtered at 4 kHz and sampled at 20 kHz. Locus coeruleus neurons were identified by the presence of a resting inwardly rectifying potassium (IRK) conductance by stepping the membrane potential from −40 to −120 mV in −10 mV increments (100 ms/step).[43] In voltage clamp experiments, neurons were maintained at −60 mV, and the series resistance was monitored with steps of −5 mV at the end of each recording. Data were discarded when the series resistance increased by >20%. The average current response was analysed off-line, and the cell capacitance (Cm) and membrane resistance (Rm) were calculated. In the current clamp mode, incremental currents from −150 to + 300 pA were injected in 25 pA steps to explore the subthreshold and firing

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properties of the neurons. Off-line analysis was performed using pClamp V9.2 (Molecular Devices, San Jose, CA).

2.8. Statistical analysis

Data are presented as mean ± SEM. The sample size was estimated using the G*Power tool to detect a significant effect using 2-tailed *t*-tests at an α level of 0.05, power of 0.80, and effect size based on the mean values from published reference studies on mechanical and thermal sensory thresholds in mice.[6,24] Statistical analysis was performed using GraphPad Prism software (GraphPad Software 9.0.3, La Jolla, CA). The Grubbs test was used to identify statistical outliers, and the Shapiro-Wilk test was used to evaluate the normality of data distribution. For comparisons between 2 groups, the unpaired Student *t* test was used for parametric data, and the Mann-Whitney *U* test for nonparametric data. One-way, two-way, or repeated-measures ANOVA followed by Tukey post hoc tests were used for comparisons among multiple groups. Area under the curve analysis was utilised for comparisons between groups across different time points. Chi-square test was employed for analysing frequency distribution. Statistical significance was set at $P < 0.05$. See detailed statistical analysis in Supplementary Tables 3 to 7, http://links.lww.com/PAIN/C454.

2.9. Data availability

All data generated during this study are available upon request.

3. Results

3.1. Behavioural sex differences in nerve-injured mice

As expected, nerve injury induced mechanical and thermal hypersensitivity in CCI-2/3w and CCI-7/11w male and female mice (cohorts #1 to #5; **Fig. 1B**; Fig. S1A, B; Supplementary Tables 1, 2, and 3, http://links.lww.com/PAIN/C454). Depressive-like behaviour assessed through the tail suspension test (TST) revealed that although CCI-2/3w mice did not show behavioural despair, both male and female CCI-7/11w mice exhibited increased immobility (cohorts #1 and #3; **Fig. 1C**) and clasping behaviours (Fig. S1C, D, http://links.lww.com/PAIN/C454) when compared with their respective sham controls (Supplementary Table 4, http://links.lww.com/PAIN/C454). Consistent findings were observed in the FST in CCI-7/11w mice (cohorts #2 and #4; Fig. S1E, F, http://links.lww.com/PAIN/C454). Correlation analyses were conducted in cohorts that underwent multiple behavioural assessments. At 2/3 weeks post-CCI, no significant correlation was observed between immobility time in the FST and acetone response duration in either sex (cohort #2; Fig. S1G, http://links.lww.com/PAIN/C454). However, by 7/11 weeks post-CCI, a significant positive correlation emerged: animals displaying prolonged responses to acetone stimulation also exhibited increased immobility in the FST (cohort #4; Fig. S1H, http://links.lww.com/PAIN/C454). These findings suggest a potential association between sensory hypersensitivity and depressive-like behaviour during the chronic phase following CCI.

Emotional impairments related to neuropathic pain were detected 7 to 11 weeks after nerve injury, prompting further assessment of emotional and cognitive behaviours at this time point. Anxiety-like behaviour, evaluated using the OF test, revealed that CCI-7/11w male mice spent significantly less time in the centre of the arena compared with sham male mice. By contrast, no differences were observed between sham and CCI-7/11w female mice (**Fig. 1D**; Supplementary Table 4, http://links.lww.com/PAIN/C454). By contrast, no differences were observed between sham and CCI-7/11w female mice (cohort #5; **Fig. 1D**; Supplementary Table 4, http://links.lww.com/PAIN/C454). Locomotor activity was similar across all groups, excluding the possibility of motor impairments (**Fig. 1E**). To rule out the possibility that all female groups had already reached a ceiling level of anxiety in this paradigm, all groups were evaluated after LPS administration, a pro-inflammatory agent and immune stressor that induces sickness behaviour accompanied by anxiety-like behaviour. To assess the impact of LPS administration, LPS-treated animals were tested in the OF test. These animals spent a reduced percentage of time in the centre zone and displayed decreased locomotor activity. The thigmotaxis ratio further confirmed an increased preference for the edges of the arena in LPS-treated animals, irrespective of sex or surgery (cohort #9; **Fig. 1F**; Fig. S2A, B, http://links.lww.com/PAIN/C454), indicating heightened anxiety-like behaviour. In the fear conditioning test, CCI-7/11w male mice developed a higher percentage of freezing during the conditioning phase compared with sham male mice, while no differences were observed in CCI-7/11w female mice. In the testing phase, neuropathic and sham animals reached the same level of conditioning (cohort #6; **Fig. 1G**). In the classical NOR task, both CCI-7/11w male and female mice exhibited impaired recognition of the novel object, indicated by a decreased DI (cohort #7; **Fig. 1H**; Fig. S2C-G, http://links.lww.com/PAIN/C454). In the NOR test assessing the ability to discriminate between 2 similar objects, both sham and CCI-7/11w male mice displayed a lower DI, reflecting a reduced ability to detect subtle differences. Although sham female mice were able to recognize the novel object, this ability was significantly diminished in CCI-7/11w female mice, evidenced by a decreased DI (**Fig. 1I**; Fig. S2H-L, http://links.lww.com/PAIN/C454).

3.2. Structural, molecular, and electrophysiological sex differences in the locus coeruleus

Immunohistochemistry (cohorts #2 and #4; **Fig. 2A**; Fig. S3A, http://links.lww.com/PAIN/C454) revealed an increase in TH+ cell density in CCI-7/11w male mice compared with sham controls. By contrast, no significant changes were detected in CCI-2/3w male mice or in female mice at either time point. (**Fig. 2B**; Fig. S3B, C, Supplementary Table 5, http://links.lww.com/PAIN/C454). Volumetric studies (**Fig. 2C**; Fig. S3D, E, http://links.lww.com/PAIN/C454) revealed similar noradrenergic somatic volume between groups along neuropathy (**Fig. 2D**). Furthermore, CCI male mice exhibited a significantly greater somatodendritic volume, attributed to an increase in dendritic volume, which persisted in both CCI-2/3w and CCI-7/11w groups. By contrast, nerve injury did not induce any changes in the LC of CCI female mice at any time point (**Figs. 2D–F**). Molecular assessments revealed no significant changes in CB1 and CRF1 receptor levels in the LC associated with neuropathic pain in either male mice or female mice (cohorts #2 and #4; **Figs. 2G and H**). Furthermore, statistical analysis of sex differences revealed a reduction in CB1 receptor levels and an increase in CRHR1 receptor levels in the LC of female mice (**Figs. 2G and H**; Supplementary Table 5, http://links.lww.com/PAIN/C454).

Whole-cell patch-clamp recordings of LC neurons were conducted in sham and CCI-7w mice. The results showed similar membrane resting potential, membrane capacitance, and resistance in both sexes (cohort #8; **Figs. 3A and B**). Resting IRK

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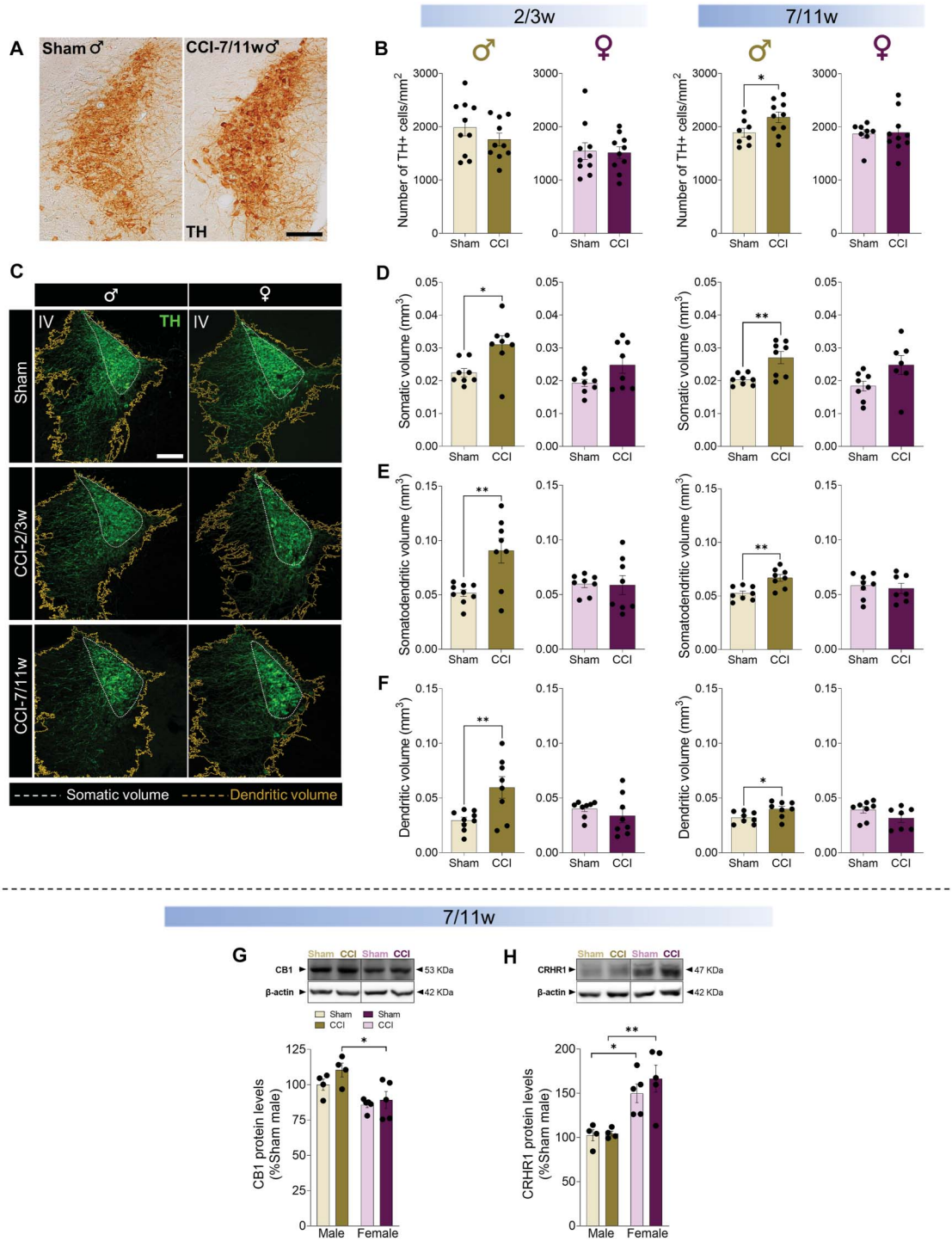

Figure 2. Quantification of TH+ cells and LC volume in male and female C57BL/6J mice after neuropathy. (A) Representative images of the LC of sham and CCI-7/11w male mice stained for TH using DAB. (B) Quantification of TH+ cell density in the LC of male and female C57BL/6J mice at 2 to 3 weeks and at 7 to 11 weeks post-surgery. (C) Representative confocal images illustrating the area occupied by the soma (white line) and dendrites (yellow line) used for volume estimation through TH immunofluorescence in the LC. (D) Volume occupied by the soma, (E) somatodendrites, and (F) dendrites of the entire LC at 2 to 3 weeks and 7 to 11 weeks post-surgery in male and female mice. (G) Western blot analysis of cannabinoid receptor 1 (CB1), (H) corticotropin-releasing hormone receptor 1 (CRHR1). Data are presented as mean ± SEM of n = 10 LC per group for DAB staining, 7 to 10 LC per group for immunofluorescence, and a pool of LC from 5 mice per group for western blot analysis: $^{*}P < 0.05$, $^{**}P < 0.01$ vs sham, Student *t* test; $^{*}P < 0.05$, $^{**}P < 0.01$, 2-way ANOVA followed by Tukey post hoc test. Scale bar = 100 μm. CCI, chronic constriction injury; DAB, 3,3′-diaminobenzidine tetrahydrochloride; IV, fourth ventricle; LC, locus coeruleus; TH, tyrosine hydroxylase.

currents were similar in sham and CCI-7w male mice but significantly higher in CCI-7w female mice compared with sham female mice (**Fig. 3C**). Further analysis of hyperpolarization and firing frequency in response to negative and positive current injections (25 pA steps) under current clamp mode revealed that CCI-7w male mice exhibited a higher firing frequency than sham male mice at the same current injection, resulting in a lower rheobase current (**Fig. 3D**). Consistent with the IRK findings,

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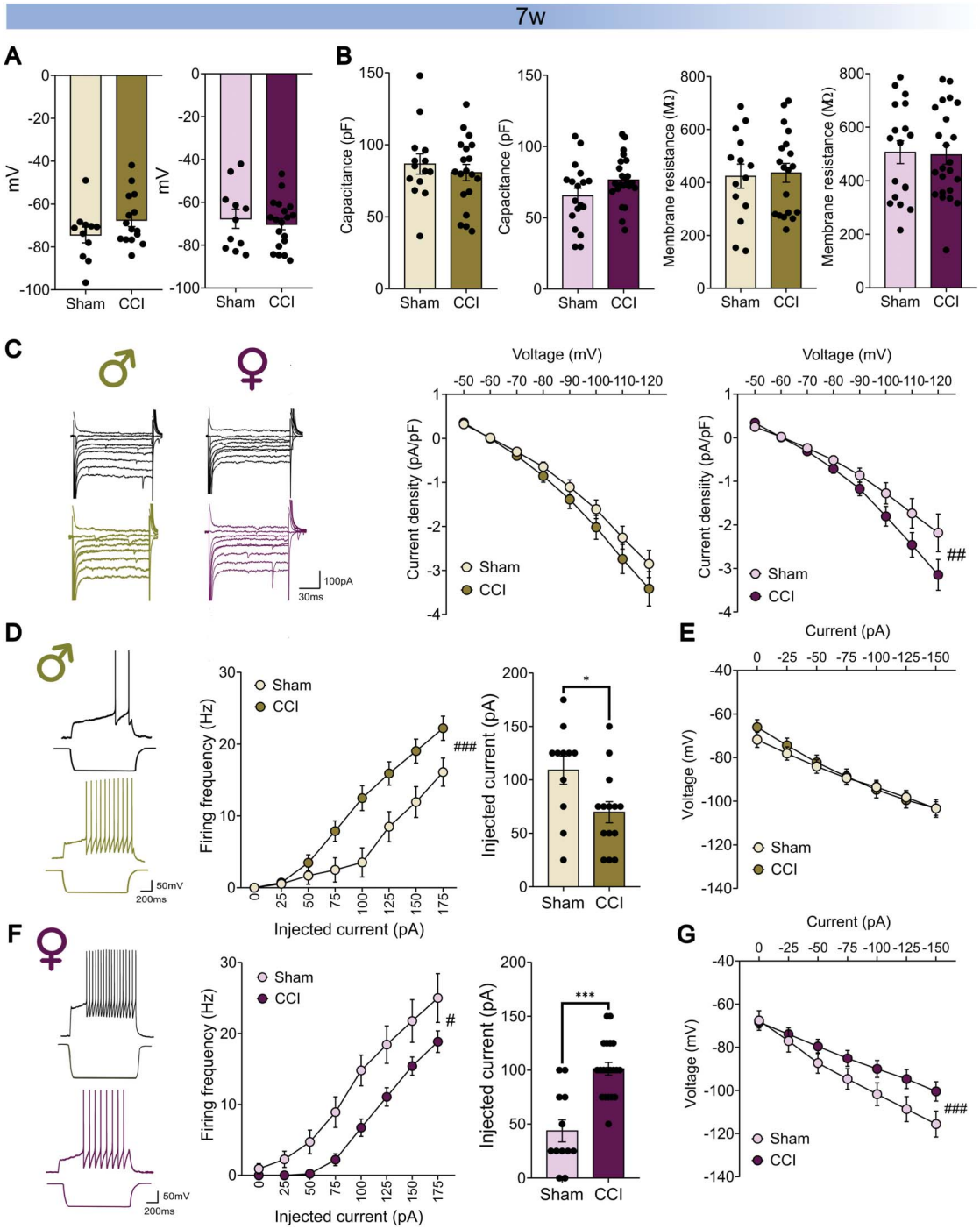

Figure 3. Evaluation of the passive properties and excitability of LC neurons in male (green) and female (purple) neuropathic mice. Graphs showing membrane resting potentials (A), membrane capacitance and resistance (B). (C) Representative examples and graphs of the IRK currents. (D) Representative examples of the voltage responses of identified LC neurons to current injection of + 100 and −100 pA, respectively, in sham and CCI-7w male mice. Graphs showing the activity driven in response to the injection of positive currents (+25 pA steps) and rheobase. (E) Graph showing the voltage deflections in response to the injection of negative currents (−25 pA steps) in sham and CCI-7w male mice. (F) Representative examples of the voltage responses of identified LC neurons to current injection of + 100 and −100 pA, respectively, in sham and CCI-7w female mice. Graphs showing the activity driven in response to the injection of positive currents (+25 pA steps) and rheobase. (G) Graph showing the voltage deflections in response to the injection of negative currents (−25 pA steps) in sham and CCI-7w female mice. Data are presented as the mean ± SEM of n = 5 to 6 mice per group: $^{*}P < 0.05$, $^{***}P < 0.001$ vs male, repeated-measures ANOVA (surgery × current factor): #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$. CCI, chronic constriction injury; IRK, inwardly rectifying potassium; LC, locus coeruleus.

hyperpolarizing steps produced similar potentials in both male groups (**Fig. 3E**). By contrast, CCI-7w female mice exhibited reduced excitability and a higher rheobase compared with sham female mices (**Fig. 3F**), along with less pronounced hyperpolarized membrane deflections (**Fig. 3G**; Supplementary Table 6, http://links.lww.com/PAIN/C454).

3.3. Sex differences in the chemogenetic inhibition of locus coeruleus neurons in nerve-injured mice

The sex-specific effects of LC-noradrenergic neuron inhibition on emotional behaviours were assessed (**Figs. 4A and B**; Fig. S4A, http://links.lww.com/PAIN/C454). Fluorescence visualization confirmed robust mCherry expression in DBH^{+} neurons, and

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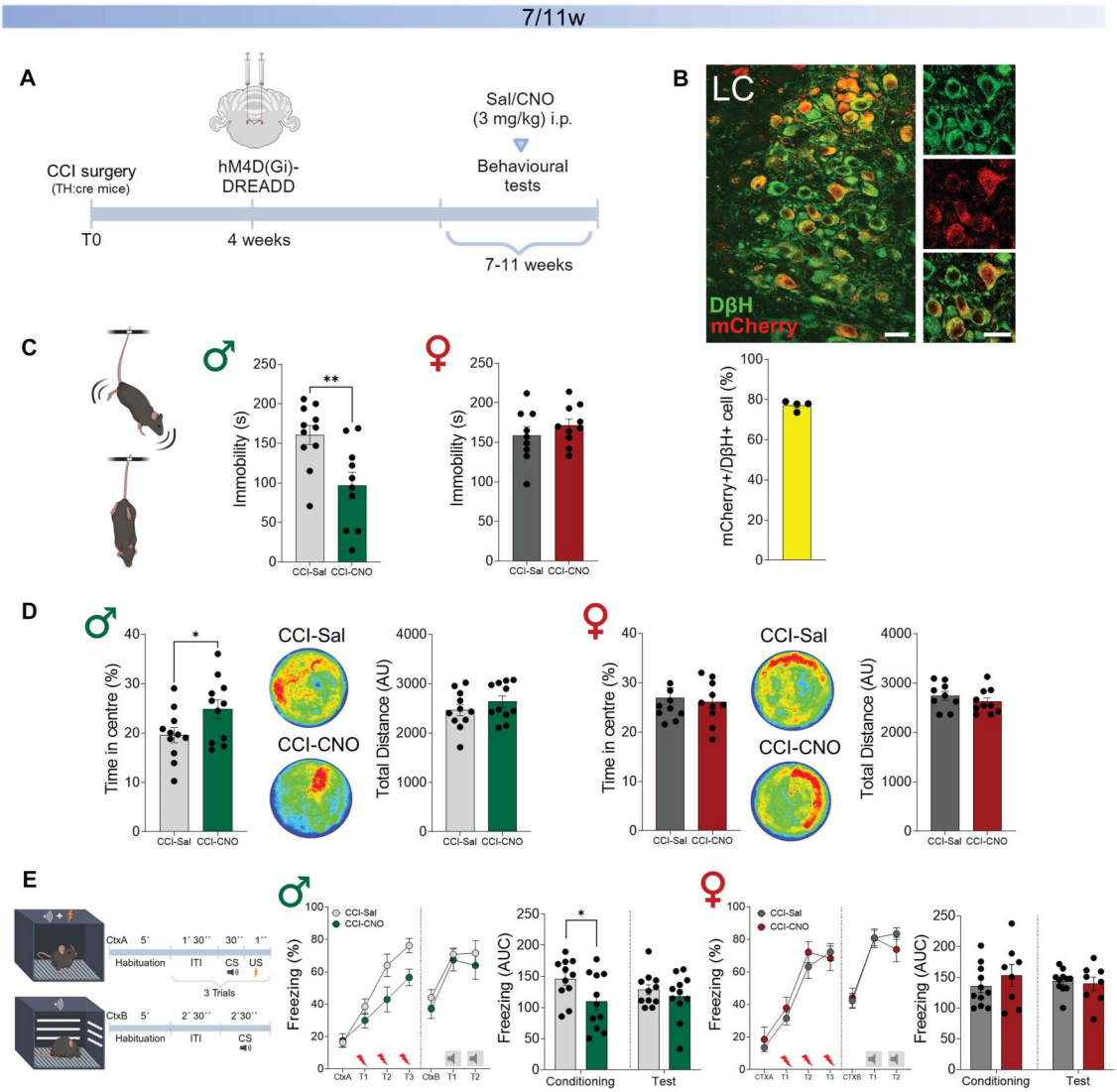

Figure 4. Effect of chemogenetic bilateral inhibition of noradrenergic neurons in the LC on depressive-like behaviour, anxiety, and fear after 7 to 11 weeks of nerve injury in male and female TH:Cre mice. (A) Design of the experimental timeline. (B) The graph shows the percentage of mCherry expression in DβH+ LC neurons. Data are presented as the mean + SEM (n = 4). Representative immunofluorescence image showing mCherry expression in noradrenergic LC neurons (red = mCherry, green = DβH) in TH:Cre mice (scale bars = 20 μm). (C) Depressive-like behaviour evaluated by the tail suspension test (TST), represented by immobility time (in seconds). (D) Anxiety-like behaviour represented as the percentage of time spent in the central zone in the open field (OF) test, along with representative heatmaps of activity, and the total distance travelled during the test (in Arbitrary Units [AU]). (E) Response to an aversive stimulus in the fear conditioning (FC) test. Schematic representation of the FC procedure for the different phases: day 1 (Context A, Conditioning) and day 2 (Context B, Test). The percentage of freezing represented during the conditioned stimulus (CS) across trials, and the area under the curve (AUC) of the percentage of freezing over time. CNO (3 mg/kg) or saline was administered intraperitoneally 30 minutes before each behavioural test. Data are presented as the mean ± SEM of $n = 8$ to 11 mice per group: $^{*}P < 0.05$, $^{***}P < 0.001$ vs CCI-CNO, Student t test. CCI, chronic constriction injury; CNO, clozapine-N-oxide; DβH, dopamine β-hydroxylase; ITI, intertrial interval; LC, locus coeruleus; US, unconditioned stimulus.

quantification in a randomly selected subset of animals (n = 4) showed that ~80% of DBH^{+} neurons were mCherry^{+} (**Fig. 4B**).

In the TST, chemogenetic inhibition reduced immobility and increased latency to immobility in CCI-7/11w male mice in the TST test, while no differences were observed in CCI-7/11w female mice (cohort #10; **Fig. 4C**; Fig. S4C, Supplementary Table 7, http://links.lww.com/PAIN/C454). Anxiety-like behaviour, assessed using the OF test, revealed that chemogenetic inhibition of the LC increased the time spent in the central zone in CCI-7/11w male mice, an effect not observed in female mice. Furthermore, locomotor activity was similar across all groups (cohort #10; **Fig. 4D**). In the fear conditioning test, chemogenetic inhibition of LC neurons resulted in a reduction of the percentage of freezing during the conditioning phase in CCI-7/11w male mice, while no differences were observed in CCI-7/11w female mice. No changes were seen in the testing phase (cohort #11; **Fig. 4E**). In sham animals, chemogenetic inhibition of the LC did not produce any behavioural changes following CNO administration (cohort #12: Fig. S5, Supplementary Table 8, http://links.lww.com/PAIN/C454). To confirm the specificity of the chemogenetic manipulation, a separate cohort of wild-type mice received CNO (3 mg/kg, i.p.); no behavioural alterations were observed in any of the tasks employed in this study (cohort #13; Fig. S6, Supplementary Table 9, http://links.lww.com/PAIN/C454), thereby supporting the specificity of the observed effects.

4. Discussion

This study examines the effects of nerve injury on the LC and related behaviours in mice, highlighting significant differences

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between sexes. Nerve injury reduced pain thresholds in both male and female mice at 2 to 3 and 7 to 11 weeks postinjury, consistent with previous data.[25,46] After evaluating the emotional sphere, we observed that male mice experiencing long-term neuropathic pain exhibited both depressive-like and anxiety-like behaviours. By contrast, female mice under the same conditions displayed depressive-like behaviour without a corresponding increase in anxiety. This aligns with findings from our systematic review and meta-analysis, which revealed a high prevalence of depression- and anxiety-like behaviours in neuropathic animals compared with sham controls, particularly in the FST and TST. However, a subgroup analysis noted substantial variation in the impact of neuropathic pain, influenced by factors such as species, strain, and model type, suggesting that specific conditions may promote the development of depression and/or anxiety-like behaviours.[17] A notable sex disparity emerged in our study, with female mice showing lower anxiety-like responses compared with male mice. Although this finding is somewhat counterintuitive given the higher prevalence of anxiety disorders and chronic pain in women,[41] it is supported by our experimental data, which showed that neuropathic male mice exhibited a higher percentage of freezing behaviour during the conditioning phase, consistent with previous studies in male neuropathic rats.[27] Conversely, neuropathic female mice did not exhibit increased anxiety-like behaviours either in OF or fear conditioning tests. These different outcomes may be attributed to inherent sex differences in stress and anxiety responses, as documented in animal and clinical studies.[3,4,7] One potential explanation is that female mice might have reached the maximum anxiogenic response detectable in the OF test. To assess this, and given that LPS is known to rapidly induce sickness behaviour accompanied by anxiety-like responses, we tested all groups in the OF following LPS administration. However, the administration of LPS effectively displays similar anxiety-like behaviour in both sexes evaluated in the open field test. Alternatively, female neuropathic mice might require strong or different stimuli to enhance anxiety or might employ alternative coping mechanisms involving distinct neural circuits for processing anxiety induced by chronic pain. Regarding non-emotional learning and memory, neuropathy led to deficits in the NOR test for both sexes, consistent with previous data. Recently, we reported that naïve female mice initially outperformed male mice in tasks requiring differentiation of objects with subtle differences.[30] However, this cognitive advantage was lost following the induction of CCI, indicating that nerve injury not only causes general cognitive deficits but also specifically impairs the advanced cognitive abilities seen in naïve female mice.

Behavioural differences between sexes may be partly attributed to sex-specific structural and functional adaptations in the LC, a sexually dimorphic key brain region involved in pain modulation and stress responses. Previous studies have demonstrated that naïve female mice possess fewer TH+ cells but greater dendritic volume in the LC, along with higher CRHR1 expression and lower CB1 protein levels.[4,30,45] These findings align with our western blot data for female sham animals, supporting the hypothesis of heightened LC excitability in female rodents. This increased excitability may reflect greater connectivity to emotion-related brain areas, potentially underlying the elevated emotional arousal and anxiety observed in naïve female mice.[30] However, following nerve injury, CRHR1 and CB1 protein levels in the LC did not differ between sexes, suggesting that other mechanisms may contribute to sex-specific adaptations. Indeed, nerve injury induced a significant increase in the somatodendritic and dendritic volume of LC neurons within 2 to 3 weeks post-neuropathy. This early structural change was followed by an increase in TH+ cell numbers and heightened LC neuron excitability at 7 to 11 weeks, consistent with previous findings.[26] By contrast, female mice showed no significant structural changes in the LC and exhibited reduced neuronal excitability in patch-clamp studies. These findings suggest that neuropathy sensitizes LC neurons in male mice while desensitizing them in female mice. Chemogenetic experiments further supported these sex differences. DREADD-mediated inhibition of LC neurons reversed neuropathic pain-induced negative consequences in male mice but had no effect on female behaviour, aligning with previous studies showing that increased LC activity impaired cognitive performance in male mice but not in female mice.[12] It is also important to consider that if pain influences TH expression, and this effect occurs differentially between male mice and female mice, vector levels could potentially vary across groups in TH: Cre transgenic animals. Therefore, conducting a more detailed and comprehensive analysis of DREADD expression among these groups could further strengthen the interpretation and offer new insights into our chemogenetic results.

These findings highlight the distinct neural mechanisms underlying emotional and cognitive responses to neuropathic pain between sexes, challenging conventional theories of female biological vulnerability to chronic pain and its emotional consequences, such as anxiety and depression.

This evidence additionally demonstrates that chemogenetic bilateral inhibition of LC-noradrenergic neurons did not alter depressive-like, anxiety, or fear-related behaviours in sham male and female mice. This may initially seem unexpected, given the well-established role of the LC in stress and affective regulation.[31,34] However, most evidence for LC involvement in negative affective states comes from studies demonstrating that increased LC activity, either globally or within specific projections, drives aversive processing. For example, McCall et al.[31] showed that optogenetic activation of LC-basolateral amygdala (BLA) projections increased norepinephrine release, neuronal excitability, and anxiety-like behaviour, while Morris et al.[34] summarized that LC activity rises during acute threat or stress. By contrast, several of our previous studies have shown that inhibition of LC activity under baseline/sham conditions has little effect on behaviour: local LC silencing with lidocaine did not change immobility in the FST,[11] global chemogenetic LC inhibition failed to affect swimming behaviour in the FST while LC activation increased immobility,[25] and inhibition of the LC-BLA pathway did not alter anxiety-like behaviour or aversive processing in sham animals.[27] Together, these findings suggest that basal LC activity is not sufficient to sustain depressive- or anxiety-like behaviour, such that its inhibition is largely behaviourally silent, whereas LC hyperactivity reliably induces negative affective states.

Our study underscores the critical need for research into sex-specific pain pathways, particularly in female mice, where mechanisms diverge markedly from those in male mice. In male mice, nerve injury endogenously activates the noradrenergic LC, leading to heightened anxiety-like behaviour and aversive learning responses, which are notably absent in female mice. This observation suggests the hypothesis that, in female nerve-injured mice, the LC could be activated through exogenous approaches (eg, optogenetics, chemogenetics, or pharmacological methods), potentially eliciting anxiety-like behaviours. This hypothesis aligns with clinical findings indicating that women exhibit lower tolerance to tricyclic antidepressants—which increase noradrenergic tone—compared with men.[23] This suggests that women with neuropathic pain may be more vulnerable to anxiety-related effects of exogenous LC activation. In

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summary, our findings emphasize the importance of adopting sex-specific strategies when targeting LC activity for chronic pain management, recognizing the distinct emotional and cognitive outcomes influenced by sex.

Conflict of interest statement

The authors have no conflict of interest to declare.

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References

[1] Aaron RV, Ravyts SG, Carnahan ND, Bhattiprolu K, Harte N, McCaulley CC, Vitalicia L, Rogers AB, Wegener ST, Dudeney J. Prevalence of depression and anxiety among adults with chronic pain: a systematic review and meta-analysis. JAMA Netw Open 2025;8:e250268.
[2] Alba-Delgado C, Mico JA, Sánchez-Blázquez P, Berrocoso E. Analgesic antidepressants promote the responsiveness of locus coeruleus neurons to noxious stimulation: implications for neuropathic pain. PAIN 2012;153:1438–49.
[3] Bangasser DA, Valentino RJ. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Front Neuroendocrinol 2014;35:303–19.
[4] Bangasser DA, Wiersielis KR, Khantsis S. Sex differences in the locus coeruleus-norepinephrine system and its regulation by stress. Brain Res 2016;1641:177–88.
[5] Bangasser DA, Zhang X, Garachh V, Hanhauser E, Valentino RJ. Sexual dimorphism in locus coeruleus dendritic morphology: a structural basis for sex differences in emotional arousal. Physiol Behav 2011;103:342–51.
[6] Barthas F, Sellmeijer J, Hugel S, Waltisperger E, Barrot M, Yalcin I. The anterior cingulate cortex is a critical hub for pain-induced depression. Biol Psychiatry 2015;77:236–45.
[7] Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 2011;35:565–72.
[8] Berrocoso E, Ikeda K, Sora I, Uhl GR, Sánchez-Blázquez P, Mico JA. Active behaviours produced by antidepressants and opioids in the mouse tail suspension test. Int J Neuropsychopharmacol 2013;16:151–62.
[9] Berrocoso E, Rojas-Corrales MO, Mico JA. Differential role of 5-HT1A and 5-HT1B receptors on the antinociceptive and antidepressant effect of tramadol in mice. Psychopharmacology (Berl) 2006;188:111–8.
[10] Busch C, Bohl J, Ohm TG. Spatial, temporal and numeric analysis of Alzheimer changes in the nucleus coeruleus. Neurobiol Aging 1997;18:401–6.
[11] Camarena-Delgado C, Llorca-Torralba M, Suárez-Pereira I, Bravo L, López-Martín C, Garcia-Partida JA, Mico JA, Berrocoso E. Nerve injury induces transient locus coeruleus activation over time: role of the locus coeruleus-dorsal reticular nucleus pathway. PAIN 2022;163:943–54.
[12] Cardenas A, Papadogiannis A, Dimitrov E. The role of medial prefrontal cortex projections to locus ceruleus in mediating the sex differences in behavior in mice with inflammatory pain. FASEB J 2021;35:e21747.
[13] Catuneanu A, Paylor JW, Winship I, Colbourne F, Kerr BJ. Sex differences in central nervous system plasticity and pain in experimental autoimmune encephalomyelitis. PAIN 2019;160:1037–49.
[14] Chandler DJ, Jensen P, McCall JG, Pickering AE, Schwarz LA, Totah NK. Redefining noradrenergic neuromodulation of behavior: impacts of a modular locus coeruleus architecture. J Neurosci 2019;39:8239–49.
[15] Colburn RW, Lubin ML, Stone DJ, Wang Y, Lawrence D, D'Andrea MR, Brandt MR, Liu Y, Flores CM, Qin N. Attenuated cold sensitivity in TRPM8 null mice. Neuron 2007;54:379–86.
[16] Curzon P, Rustay NR, Browman KE. Cued and contextual fear conditioning for rodents. CRC Press / Taylor & Francis Group, LLC, In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. Boca Raton, FL, 2009.
[17] de la Rosa T, Llorca-Torralba M, Martinez-Cortes A, Romero-Lopez-Alberca C, Berrocoso E. A systematic review and meta-analysis of anxiety- and depressive-like behaviors in rodent models of neuropathic pain. Biol Psychiatry Glob Open Sci 2024;4:100388.
[18] Emery EC, Young GT, Berrocoso EM, Chen L, McNaughton PA. HCN2 ion channels play a central role in inflammatory and neuropathic pain. Science 2011;333:1462–6.
[19] Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. PAIN 1988;32:77–88.
[20] INFRAFRONTIER Consortium. INFRAFRONTIER–providing mutant mouse resources as research tools for the international scientific community. Nucleic Acids Res 2015;43:D1171–5.
[21] Joshi N, Chandler D. Sex and the noradrenergic system. Handb Clin Neurol 2020;175:167–76.
[22] Kasai S, Yamamoto H, Kamegaya E, R Uhl G, Sora I, Watanabe M, Ikeda K. Quantitative detection of μ opioid receptor: western blot analyses using μ opioid receptor knockout mice. Curr Neuropharmacol 2011;9:219–22.
[23] LeGates TA, Kvarta MD, Thompson SM. Sex differences in antidepressant efficacy. Neuropsychopharmacology 2019;44:140–54.
[24] Li J, Wei Y, Zhou J, Zou H, Ma L, Liu C, Xiao Z, Liu X, Tan X, Yu T, Cao S. Activation of locus coeruleus-spinal cord noradrenergic neurons alleviates neuropathic pain in mice via reducing neuroinflammation from astrocytes and microglia in spinal dorsal horn. J Neuroinflammation 2022;19:123.
[25] Llorca-Torralba M, Camarena-Delgado C, Suárez-Pereira I, Bravo L, Mariscal P, Garcia-Partida JA, López-Martín C, Wei H, Pertovaara A, Mico JA, Berrocoso E. Pain and depression comorbidity causes asymmetric plasticity in the locus coeruleus neurons. Brain 2022;145:154–67.
[26] Llorca-Torralba M, Pilar-Cuéllar F, Bravo L, Bruzos-Cidon C, Torrecilla M, Mico JA, Ugedo L, Garro-Martínez E, Berrocoso E. Opioid activity in the locus coeruleus is modulated by chronic neuropathic pain. Mol Neurobiol 2019;56:4135–50.
[27] Llorca-Torralba M, Suárez-Pereira I, Bravo L, Camarena-Delgado C, Garcia-Partida JA, Mico JA, Berrocoso E. Chemogenetic silencing of the locus Coeruleus-Basolateral Amygdala Pathway abolishes pain-induced

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anxiety and enhanced aversive learning in rats. Biol Psychiatry 2019;85:1021–35.
[28] Luque JM, de Blas MR, Segovia S, Guillamón A. Sexual dimorphism of the dopamine-β-hydroxylase-immunoreactive neurons in the rat locus ceruleus. Develop Brain Res 1992;67:211–5.
[29] MacDowell KS, Sayd A, García-Bueno B, Caso JR, Madrigal JLM, Leza JC. Effects of the antipsychotic paliperidone on stress-induced changes in the endocannabinoid system in rat prefrontal cortex. World J Biol Psychiatry 2017;18:457–70.
[30] Mariscal P, Bravo L, Llorca-Torralba M, Razquin J, Miguelez C, Suárez-Pereira I, Berrocoso E. Sexual differences in locus coeruleus neurons and related behavior in C57BL/6J mice. Biol Sex Differ 2023;14:64.
[31] McCall JG, Siuda ER, Bhatti DL, Lawson LA, McElligott ZA, Stuber GD, Bruchas MR. Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior. Elife 2017;6:e18247.
[32] Michel RP, Cruz-Orive LM. Application of the Cavalieri principle and vertical sections method to lung: estimation of volume and pleural surface area. J Microsc 1988;150:117–36.
[33] Miguelez C, Morin S, Martinez A, Goillandeau M, Bezard E, Bioulac B, Baufreton J. Altered pallido-pallidal synaptic transmission leads to aberrant firing of globus pallidus neurons in a rat model of Parkinson's disease. J Physiol 2012;590:5861–75.
[34] Morris LS, McCall JG, Charney DS, Murrough JW. The role of the locus coeruleus in the generation of pathological anxiety. Brain Neurosci Adv 2020;4:239821282093032.
[35] Mulvey B, Bhatti DL, Gyawali S, Lake AM, Kriaucionis S, Ford CP, Bruchas MR, Heintz N, Dougherty JD. Molecular and functional sex differences of noradrenergic neurons in the mouse locus coeruleus. Cell Rep 2018;23:2225–35.
[36] Ohm TG, Busch C, Bohl J. Unbiased estimation of neuronal numbers in the human nucleus coeruleus during aging. Neurobiol Aging 1997;18:393–9.
[37] Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. London, United Kingdom: Elsevier; 2019.
[38] Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–2.
[39] Rivera B, Moreno C, Lavanderos B, Hwang JY, Fernández-Trillo J, Park KS, Orio P, Viana F, Madrid R, Pertusa M. Constitutive phosphorylation as a key regulator of TRPM8 channel function. J Neurosci 2021;41:8475–93.
[40] Suárez-Pereira I, Carrión Á. Updating stored memory requires adult hippocampal neurogenesis. Sci Rep 2015;5:13993.
[41] Tsang A, Von Korff M, Lee S, Alonso J, Karam E, Angermeyer MC, Borges GL, Bromet EJ, Demytteneare K, de Girolamo G, de Graaf R, Gureje O, Lepine JP, Haro JM, Levinson D, Oakley Browne MA, Posada-Villa J, Seedat S, Watanabe M. Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. J Pain 2008;9:883–91.
[42] Valentino RJ, Bangasser DA. Sex-biased cellular signaling: molecular basis for sex differences in neuropsychiatric diseases. Dialog Clin Neurosci 2016;18:385–93.
[43] Williams JT, North RA, Tokimasa T. Inward rectification of resting and opiate-activated potassium currents in rat locus coeruleus neurons. J Neurosci 1988;8:4299–306.
[44] Wurzman R, Forcelli PA, Griffey CJ, Kromer LF. Repetitive grooming and sensorimotor abnormalities in an ephrin-A knockout model for Autism Spectrum Disorders. Behav Brain Res 2015;278:115–28.
[45] Wyrofsky RR, Reyes BAS, Yu D, Kirby LG, Van Bockstaele EJ. Sex differences in the effect of cannabinoid type 1 receptor deletion on locus coeruleus-norepinephrine neurons and corticotropin releasing factor-mediated responses. Eur J Neurosci 2018;48:2118–38.
[46] Yalcin I, Bohren Y, Waltisperger E, Sage-Ciocca D, Yin JC, Freund-Mercier MJ, Barrot M. A time-dependent history of mood disorders in a murine model of neuropathic pain. Biol Psychiatry 2011;70:946–53.

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