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Prepulse inhibition can predict the motivational effects of cocaine in female mice exposed to maternal separation

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ABSTRACT

The prepulse inhibition (PPI) of the startle response can identify the rodents that are more sensitive to the effects of cocaine. Mice with a lower PPI presented a higher vulnerability to the effects of cocaine and a higher susceptibility to developing a substance use disorder (SUD). Maternal separation with early weaning (MSEW) is a relevant animal model to induce motivational alterations throughout life. Nevertheless, only a few studies on females exist, even though they are more vulnerable to stress- and cocaine-related problems. Hence, the aim of the present study was to evaluate the ability of PPI to identify females with a greater vulnerability to the long-term consequences of early stress on the motivational effects of cocaine. Female mice underwent MSEW and were classified according to their high or low PPI. They were then assessed in the cocaine-induced locomotor sensitization test, the conditioned place preference paradigm or the operant self-administration paradigm. Additionally, they were also evaluated in the passive avoidance task, the tail-suspension and the splash tests. The results revealed that the females with lower PPI presented higher consequences of MSEW on the effects of cocaine and showed an increase in anhedonia-like behaviours. Our findings support that a PPI deficit could represent a biomarker of vulnerability to the effects of cocaine induced by MSEW.

1. Introduction

Cocaine is the most widely used illegal psychostimulant drug in North America and Europe, primarily consumed by young adults [1,2]. Cocaine addiction is characterized by a high and persistent susceptibility to relapse [3], and its compulsive use is a serious public health problem with an important social and economic impact [4–6]. Additionally, there is no approved treatment deemed effective for dependence on this drug [7,8].

Recent reports reveal an increased trend in cocaine users among several groups during the last decade, mainly females and the 18–25 age range [2,9]. Although cocaine use is much greater in men than in women [2,10], the increased trend among females is particularly concerning, as there seems to be a higher vulnerability to cocaine-related problems in women users than in men [9]. In fact, the reinforcing effects of cocaine are more powerful in the female population than in males. Females show faster and greater cocaine seeking and intake of the drug, evolving more rapidly from drug use to abuse [11–15]. Moreover, the side effects of drug use are greater for women, who are more likely to relapse than men [11,16]. Additionally, it has been suggested that early adversities during childhood increase women's vulnerability to relapse [12]. These stressful situations during the early stages of development could be an important factor for the onset of psychiatric disorders, such as depression and substance use disorders (SUD) [17–19]. In spite of this, studies with females are still scarce [20], despite the fact that women seem to present a higher vulnerability to cocaine addiction.

During the last decades, several rodent behavioural models on early life stress, such as maternal separation, have been used to study the neurobiological basis of emotional and cognitive disorders, including SUD [21]. In this sense, maternal separation with early weaning (MSEW) is a reliable animal model of childhood adversity [22–26], causing long-term behavioural alterations in rodents such as

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depressive-like behaviour [19,21,26–29]. Specifically, it has been observed that MSEW increases anhedonia-like behaviour and changes in sensitivity to the conditioned reinforcement of cocaine and alcohol in adolescent and adult male mice, respectively [23,25,28,30]. Some of these alterations have been related to the development of an SUD in humans. In addition, despite women showing more pathologies associated with chronic stress exposure [31], there are very few studies evaluating the effects of the early stressor on females [24]. For this reason, it is necessary to identify psychological and physiological biomarkers of early vulnerability factors leading to cocaine consumption, such as endophenotypes [32], to identify the individuals that are most vulnerable to developing a cocaine use disorder.

In this sense, our laboratory has previously used the prepulse inhibition paradigm (PPI) to discriminate subpopulations with different vulnerabilities to cocaine effects [33,34]. The prepulse inhibition (PPI) of the startle response is an operational measure of sensory-motor gating, which may indicate alterations in the cerebral dopaminergic system, such as the mesolimbic pathway [35]. A deficit in PPI is considered an endophenotype of psychotic symptoms, and it has been observed in other psychiatric disorders including schizophrenia, obsessive compulsive disorder, Gilles de la Tourette's syndrome, bipolar mania and high functioning autism [36,37]. Recently, it has been reported that the PPI score of mice can predict their sensitivity to the conditioned rewarding and motor effects of cocaine [33,34]. Thus, male and female mice with a low PPI presented a lower sensitivity to the conditioned reinforcing effects and motor effects of the drug than those with a high PPI. Nevertheless, these Low-PPI mice showed stronger associative cocaine effects with environmental cues when they were exposed to higher doses of cocaine [33] and they were more vulnerable to develop behavioural sensitization induced by the drug when they were exposed to an intermittent administration of cocaine [34]. Accordingly, PPI sensitivity can be useful to discriminate between animals with a higher vulnerability to the effects of cocaine, who are more likely to display behaviours induced by the drug, and which in turn can increase the risk of developing a cocaine use disorder.

The aim of the present study was to evaluate the predictive ability of PPI to identify animals with a greater vulnerability to use cocaine after exposure to MSEW. For this purpose, adult female mice that underwent MSEW or Standard Nest (SN) were first tested in the PPI paradigm in order to classify them as High or Low PPI. Afterwards, the motivational effects of cocaine were assessed in different groups of mice, using the cocaine-induced locomotor sensitization test (Experiment 1), the conditioned place preference (CPP) paradigm (Experiment 2) and the operant self-administration (SA) paradigm (Experiment 3). These experiments were designed to explore the whole profile of vulnerability to cocaine in three different paradigms (locomotor, context and motivation) depending on the PPI presented. Moreover, we also investigated differences between High or Low PPI females in the emotional and depressive-like behaviours induced by early stress, such as passive avoidance task, tail-suspension and splash tests, which can help to comprehend the differences between High or Low PPI mice in the long-term consequences of stress on the response to cocaine.

2. Material and methods

2.1. Animals

A total of 221 female mice of the CD1 strain (Charles River, Francia) were employed. Fourteen male and fourteen female adult mice used as breeders were received at 8 weeks of age at the animal facility, UBIO-MEX, PRBB. On arrival, breeding pairs were housed in standard cages in a controlled temperature (21 ± 1 °C) and humidity (55 % ± 10 %) under a 12 h light/dark cycle (lights on from 20:00 to 8:00), with ad libitum access to food and water. After ten days, male mice were removed, and pregnant females were housed individually.

2.2. Rearing conditions

Mice were randomly assigned to one of the two different experimental groups, SN or MSEW animals. The maternal separation protocol was conducted as reported by Gracia-Rubio et al. [23,24] and Portero-Tresserra et al. [25]. Briefly, pregnant females were observed daily at 9:00 and 17:00 for parturition. For each litter, the date of delivery was assigned as postnatal day (PND) 0. In the MSEW group, offspring were separated from their mothers for 4 h per day on PND 2-5 (9:00–13:00) and 8 h per day on PND 6-16 (9:00–17:00). For separation, mothers were moved to another cage, while the offspring remained in their home cages with a heating blanket (32–34 °C) for thermoregulation. Pups in the MSEW group were early weaned on PND 17, while pups in the SN group remained with their mothers until the day of weaning (PND 21). In both cases (SN and MSEW), the cages were cleaned on PND 10. After weaning, females were housed undisturbed (except for cleaning) in groups of 5. We have distributed the pups of each litter between the different experimental groups to avoid a litter effect. We used less than 25 % of the animals from the same litter in each group. Males were used in other experiments.

2.3. Drugs

Cocaine HCl was purchased from Alcatel (Ministry of Health, Madrid, Spain) and was dissolved in sterile physiological saline (0.9 %, NaCl solution). The dose of cocaine used for the acquisition phase of the self-administration (SA) procedure was 1 mg/Kg/infusion (i.v.); for conditioned place preference 1 and 12 mg/Kg (i.p.); and for locomotor sensitization test 10 and 25 mg/kg (i.p.).

2.4. Apparatus and procedure

Fig. 1 shows (a) experimental groups and timelines and (b) the specific procedure followed in each experiment.

2.4.1. Prepulse inhibition (PPI)

2.4.1.1. Apparatus. Two startle measuring devices were used, consisting of a Plexiglas tube (28 × 15 × 17 cm) on top of a platform with a sensor on its base that allows measuring the force that the animal exerts onto the platform after a sound stimulus is heard. The value used in the study is the peak value of the startle response. This value is transduced by an accelerometer, the signal is collected, digitized and presented on the computer. The apparatus (mod startle response CERS) and program were purchased from CIBERTEC, S.A, Madrid. Spain. The description of the unit is found in Arenas et al. [33].

2.4.1.2. Procedure. The PPI procedure was performed between PNDs 42–49 in three sets of animals (Experiment 1, n = 101; Experiment 2, n = 51; Experiment 3, n = 69). Based on Valsamis & Schmid's [38] and previous work [33], the experiment was carried out in two different phases in two consecutive days. The first day was the acclimation phase. Mice were placed into the animal holder for 5 min with a constant 65-dB white noise as background noise without startle stimuli. During this session, the animal would calm down, stop exploring the environment and stop moving around. The animal holder was cleaned after an animal was removed. In the second phase, the PPI was evaluated. This phase also started with 5 min of 65 dB white noise followed by a programme of stimuli while the white noise kept playing in the background. The programme consisted of two parts: the first one was a series of 50 trials of pulses of 120 dB to establish the habituation of startle. The 120 dB stimulus was chosen as the maximum value reached in the startle response in pilot studies. In the second part, two different prepulse intensities (75 and 85 dB during 4 ms each) were used with two different inter-stimulus intervals (30 and 100 ms) and one single pulse at an

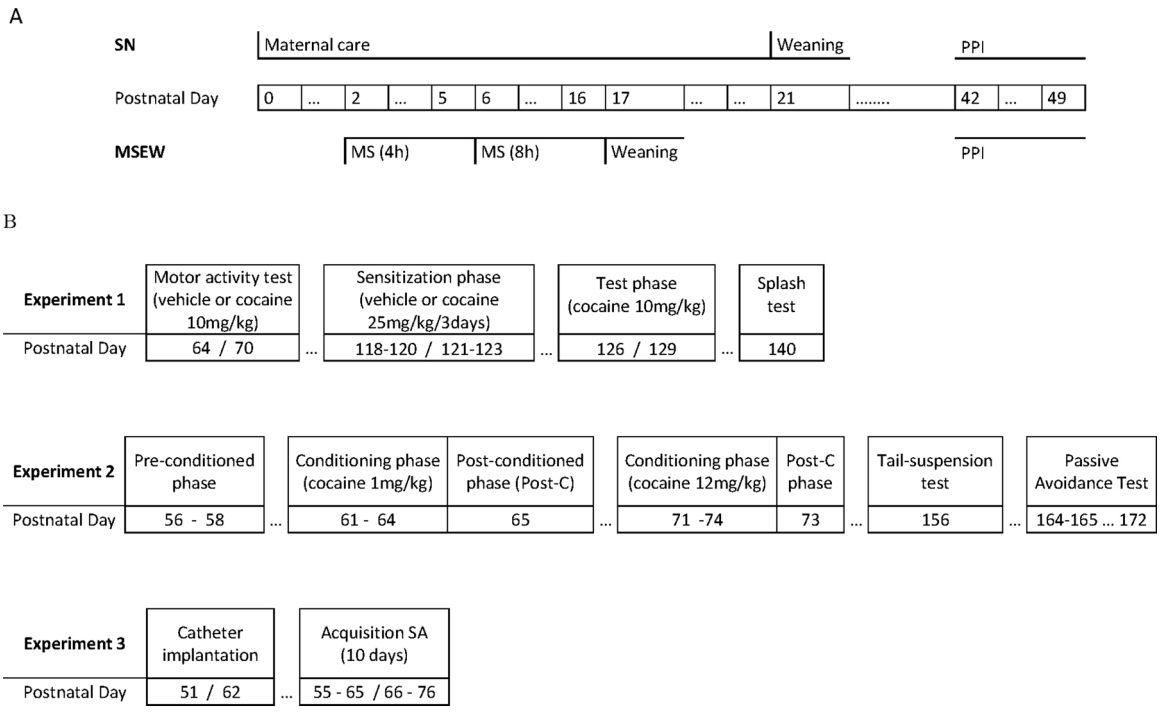

Fig. 1. Experimental procedure. A) Experimental groups and timelines. B) Specific procedures in each set of mice.

intensity of 120 dB during 20 ms each to calculate the baseline startle. Thus, there were four types of prepulse trials: 75 dB/30 ms, 75 dB/100 ms, 85 dB/30 ms and 85 dB/100 ms; all followed by a 120-dB pulse. The four types of trials were run 10 times alongside single instances of the 75, 85 and 120 dB tones, each in pseudorandom order, totalling 70 trials with a 20-s duration for each trial. The prepulse-alone trials (75/85 dB) were introduced to verify that they were not acting as pulses and to confirm that only the 120-dB pulse was the main stimulus to induce the startle response in the animal. The total time for the test was 45 min.

The PPI was calculated as a percentage score: PPI(%) = 100 - (startle response for pulse with pre - pulse × 100/startle response for pulse alone), and the mean of the four PPI obtained (75 dB/30 ms, 75 dB/100 ms, 85 dB/30 ms and 85 dB/100 ms) was used to divide the animals according to their high or low PPI, performing a two-cluster analysis of K mean with SN mice. The categorization of MSEW animals as high or low PPI was determined using the results of the two-cluster analysis of K mean on the SN mice, since MSEW animals had already been subjected to a stressful situation that could have affected their PPI.

2.4.2. *Experiment 1. Cocaine effects on locomotion*

2.4.2.1. *Motor activity and locomotor sensitization*

2.4.2.1.1. Apparatus. Motor activity was measured by an actimeter (CIBERTEC SA, Spain) consisting of eight cages (33 × 15 × 13 cm), with infrared lights located around the cage on the horizontal axis and 2 cm away from the bottom of the cage, which coincides with the body of the mice. It allows the automatic registration of the locomotor displacement (horizontal) and the motor activity (vertical).

Animals were injected to assay motor activity induced by an acute cocaine dose (10 mg/kg) on PNDs 64–70; to induce sensitization on PNDs 118–123; and, to evaluate the challenge test, on PNDs 126−130.

2.4.2.1.2. Motor activity procedure. To assess the acute motor effects of cocaine or motor sensitization induced by the drug, we first recorded the animal's activity for 60 min before administering cocaine, during the phase of habituation to the environment. After the habituation period, we administered an injection of cocaine (10 mg/kg) or vehicle to evaluate the motor activity during 10-min intervals in a 30-min session in the test phase.

2.4.2.1.3. Locomotor sensitization procedure. The cocaine motor sensitization was evaluated following the protocol previously described by Ferrer-Pérez et al. [39]. The motor sensitization schedule is divided into three phases. Briefly, the three phases consist of: i) the sensitization induction phase induced by a daily injection of cocaine (25 mg/kg) or saline during three consecutive days; ii) the sensitization development phase consisting of an interval of 5 consecutive days with no injections administered; and iii) test phase, induced by a single administration of 10 mg/kg of cocaine (the challenge test), where the motor activity of all the mice is evaluated.

2.4.2.2. Splash test. After testing motor activity and locomotor sensitization to cocaine, the splash test was performed. It consisted of spraying a 10 % sucrose solution on the dorsal coat of mice, which were habituated to the room for 1 h before testing, following the protocol previously described by Hodes et al. [40]. The total time of grooming over a 5-minute period was recorded and scored by blind trained observers. The percentages of time spent by the animal's leg- and back-grooming were analysed.

2.4.3. *Experiment 2. Cocaine-induced conditioned place preference (CPP)*

2.4.3.1. *Conditioned place preference*

2.4.3.1.1. Apparatus. The apparatus consisted of twelve identical Plexiglas place-conditioning boxes. These boxes consist of two equally sized compartments (30.7 cm × 31.5 cm × 34.5 cm) separated by a grey central area (13.8 cm × 8 cm × 34.5 cm). The compartments of this apparatus have different coloured walls (black vs. white) and distinct floor textures (smooth in the black compartment and rough in the white). Four infrared light beams in each of the box's compartments and six in the central area allow the position of the animal and its crossings from one compartment to the other to be recorded. The equipment was controlled by an IBM PC computer using MONPREZ software (CIBERTEC, SA, Spain).

2.4.3.1.2. Procedure. The CPP procedure is unbiased in terms of initial spontaneous preference, and it was performed as previously

described [41,42]. In short, in the first phase (pre-conditioning/Pre-C), mice were allowed access to all three compartments of the apparatus for 15 min (900 s) per day over two days. On Day 3 (PND 60), the time spent in each compartment during a 900-s period was recorded. Some animals showed a strong unconditioned aversion (less than 27 % of the session time; i.e., 250 s) or preference (more than 73 % of the session time; i.e., 650 s) for a given compartment and were therefore discarded from the rest of the experimental procedure. In each group, half of the animals received the drug or vehicle in one compartment and the other half received it in the other compartment. The mice were randomly assigned to the conditioning compartment. An ANOVA analysis revealed no significant differences between the time spent in the drug-paired and vehicle-paired compartments during the pre-conditioning phase. This is an important step in the experimental procedure that avoids any preference bias prior to conditioning. In the second phase (conditioning), which lasted 4 days, animals received an injection of physiological saline immediately before being confined to the vehicle-paired compartment for 30 min. After an interval of 4 h, they received an injection of cocaine (1 or 12 mg/kg) immediately before being confined to the drug-paired compartment for 30 min. Confinement was carried out in both cases by closing the guillotine door that separated the two compartments. During the third phase, known as post-conditioning (Post-C), the guillotine door separating the two compartments was removed (day 8) and the time spent by the untreated mice in each compartment during a 900-s observation period was recorded. The difference in seconds between the time spent in the drug-paired compartment during the Post-C and the Pre-C phases is a measure of the degree of conditioning induced by the drug. If this difference is positive, then the drug has induced a preference for the drug-paired compartment, while the opposite indicates that an aversion has developed.

Groups showing CPP in Post-C underwent an extinction session, twice per week, during which they were placed in the apparatus (without the guillotine doors separating the compartments) for 15 min. Extinction was confirmed when, in two consecutive sessions, the time spent by each group in the drug-paired compartment was similar to that of Pre-C and different from that of Post-C (Student's *t*-test). When extinction had been confirmed in an additional session (24 h after), a reinstatement test was performed with half the dose of cocaine. Reinstatement tests were the same as those carried out in Post-C (free ambulation for 15 min), except that animals were tested 15 min after administration of the respective dose of cocaine. When reinstatement of the preference was achieved by a subsequent extinction process, a new reinstatement test was conducted with progressively lower doses of the drug (half the dose at each successive reinstatement). The complete CPP procedure was performed between the PND 49–88.

2.4.3.2. Tail suspension test. In the tail suspension test, each mouse was suspended individually (using adhesive tape attached 1 cm from the tip of the tail) 50 cm above a benchtop for 6 min. The time spent immobile by the animal during this interval was recorded and scored by an observer blind to the experimental conditions [43].

2.4.3.3. Passive avoidance task

2.4.3.3.1. Apparatus. We employed a passive avoidance apparatus for mice (Ugo Basile, Comerio-Varese, Italy), consisting of a Perspex cage divided into two sections (15 × 9.5 × 16.5 cm each). The chambers were separated by a flat-box partition consisting of an automatically operated sliding door at floor level. A light (24 V, 10 W) in the ceiling of the starting compartment was left switched on during the experiment while the other side was kept in darkness. The floor was composed of stainless steel bars, 0.7 mm in diameter and 8 mm apart.

2.4.3.3.2. Procedure. The passive avoidance task was evaluated following the protocol previously described by Navarro-Francés and Arenas [44]. The training session (or training) began with a 60-s period of acclimatization to the box, during which the door separating the two compartments was kept closed. After this period, the door opened automatically. When the animal crossed from the light compartment to the dark one, the closed door and the animal received a 0.5 mA shock during a 3-s period. The time the animal took to cross from one compartment to another was recorded (crossing latency). The animal was allowed a maximum of 5 min to cross from the light to the dark compartment after which the trial terminated. In the test session, which began 24 h later, each animal was placed once again in the illuminated compartment for an adaptation period of 60 s. Afterwards, the door opened and the time the animal took to enter the chamber in which it had previously received the shock was recorded. No shock was administered on this occasion. The animal was allowed a maximum of 5 min to enter the dark chamber, after which, the test terminated. If it failed to cross the chamber, it was placed by the experimenter in the black compartment for a 5-s period without any shock being administered. After one week, the retention session was carried out following the same protocol as the test session in order to evaluate the retention of the conditioned response in each animal. The latencies to cross in the training test and retention sessions of passive avoidance conditioning were analysed.

2.4.4. Experiment 3. Operant cocaine self-administration

2.4.4.1. Apparatus. The self-administration experiments were carried out in mouse operant chambers (Model ENV-307A-CT, Medical Associates, Cibertec S.A., Madrid Spain) containing two holes: one defined as active and the other as inactive. Nose poking into the active hole delivered a cocaine infusion (reinforcement) that was paired with two stimuli light, one placed inside the nose-poke hole and the other above the active hole. Nose poking into the inactive hole had no consequences. Active/inactive holes were randomly assigned to either side and counterbalanced. The chambers were placed in sound- and light-attenuated boxes with fans to provide ventilation and white noise.

2.4.4.2. Surgery for the catheter implantation. After the PPI, all the animals (SN = 40; MSEW = 40) were implanted with a catheter to allow self-administration at PND 51−62. The surgical procedure was performed as described previously [45–47]. In short, animals were anesthetized with a mixture of ketamine/xylazine (50 mg/mL, 10 mg/mL, administered by intraperitoneal route in a volume of 0.15 mL/10 g), and then implanted with the catheter in the jugular vein. Animals were treated with analgesics (meloxicam 0.5 mg/kg, i.p., administered in a volume of 0.10 mL/10 g) and antibiotic solution (Enrofloxacin 7.5 mg/kg, i.p., administered in a volume of 0.03 mL/10 g). After surgery, animals were housed individually and laid over electric blankets to facilitate their recovery.

2.4.4.3. Acquisition. At least 3 days after surgery, females were trained on a fixed ratio 1 (FR1) to self-administer cocaine (1.0 mg/kg per infusion) during 10 consecutive days (2 h/day). Cocaine infusion was delivered in 20 μL over 2 s via a syringe located in a microinfusion pump (PHM-100A, Med-Associates, Georgia, VT, USA) and connected via Tygon tubing (0.96 mm outer diameter, Portex Fine Bore Polythene Tubing, Portex Limited, Kent, England) to a liquid swivel (375/25, Instech Laboratories, Plymouth Meeting, PA, USA) and the mouse intravenous (iv) catheter. In order to avoid overdosing, mice received a maximum of 150 infusions and each infusion was followed by a 15-s time-out period in which no cocaine infusions were delivered. At the beginning of each session, the house light was on for 3 s and off during the rest of the experiment. The session started with a cocaine priming injection and a 4-s presentation of the light cue, situated above the active hole. The number of infusions (responses during time in) in the active and the inactive holes were counted. The criteria for the acquisition of a stable SA were: 5 or more responses in the active hole, more than 65 % of responses in the active hole, and a stable response during two

consecutive days. The patency of the iv catheters was evaluated at the end of the acquisition phase via the infusion of 0.1 mL of tiobarbital (thiopental sodium; 5 mg/mL; iv; B. Braun Medical, S.A. Rubí, Barcelona, Spain). When signs of anaesthesia did not appear within the first 3 s, the mouse was removed from the experiment.

2.5. Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics v23.0 software. For the distribution of the female mice according to their higher or lower PPI, a cluster analysis of K media was performed as previously described in the procedure. For the locomotor activity and sensitization data, a repeated measures ANOVA with three between variables, Rearing (SN and MSEW), PPI (High and Low level) and Drug or Pre-treatment (Saline and Cocaine), and one within variable, Time (four 10-min periods). In order to test our hypothesis about varying vulnerability to the effects of cocaine in Low- and High-PPI mice, we also performed the Tukey's honest significant difference (HSD) test, which does not require previous ANOVA with a significant interaction between factors and is highly conservative against type I error [48]. For the CPP data, the time spent in the drug paired compartment during the Pre- and Post-C tests was analysed with a repeated measures ANOVA, with two between variables, Rearing (SN and MSEW) and PPI (High and Low level) and one within variable, Days (Pre-Conditioning and Post-Conditioning). CPP was considered to have been established when the time spent in the drug-paired compartment during the Post-C test was $\geq$ 50 s longer than that of pre-conditioning test [49]. In order to evaluate the extinctions and reinstatements of the conditioned preference, different Student's t-tests were performed for each experiment. The time required for the preference to be extinguished in each animal was analysed by means of the Kaplan–Meier test. For the self-administration data, the number of infusions were analysed using a four-way repeated measures ANOVA with Days (day 1 to day 10), Hole (active and inactive) as intra-subject variables, and PPI (High and Low level) and Rearing (SN and MSEW group) as inter-subject variables. To evaluate the total intake of cocaine, a two-way ANOVA with Rearing (SN and MSEW) and PPI (High and Low level) was performed. For the percentage of acquisition, the Chi square test was employed. For the data of the tail suspension and splash tests, an ANOVA with two between variables, Rearing (SN and MSEW) and PPI (High and Low level), was performed for each measure. For the passive avoidance data, an ANOVA with repeated measurements, with two between variables, Rearing (SN and MSEW) and PPI (High and Low level), and one within variable, Sessions (Training, Test and Re-Test), was used to evaluate the crossing latency to the dark compartment. When required, the ANOVA analysis was followed by Bonferroni's pairwise post-hoc comparisons. In order to describe the relations between the PPI score and other variables, linear regression analyses were performed. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Experiment 1. Consequences of MSEW on the motor effects of cocaine

SN mice were classified as High- or Low-PPI animals using a two-cluster analysis [$F(1,46) = 91.827$; $p < 0.0001$]. Afterwards, MSEW females were categorized as high or low PPI using the data obtained from the two-cluster analysis of K mean performed on the SN mice (High-PPI: from 16.07 to 61.62; Low-PPI: from -43.71 to 13.99). SN and MSEW females did not show significant differences in their PPI scores [$F(1,99) = 0.269$; $p < 0.605$] (SN Low-PPI: -2.4 n = 20; SN High-PPI: 30.91 n = 28; MSEW Low-PPI: -2.5 n = 25; MSEW High-PPI: 30.4 n = 28).

The ANOVA of motor activity induced by acute cocaine 10 mg/kg (Fig. 2A and B) showed a significant main effect of the variables Time [$F(3,89) = 18.024$; $p < 0.0001$], and Drug [$F(1,91) = 30.503$; $p < 0.0001$]. Regarding two-way interactions, Time x Drug [$F(3,89) = 9.153$; $p < 0.0001$] and Time x Rearing were significant [$F(3,89) = 4.751$; $p < 0.004$]. Finally, the four-way interaction Time x Rearing x PPI x Drug did not reach statistical significance, but the Tukey HSD test indicated that SN High-PPI females treated with cocaine presented higher motor activity than SN High-PPI females treated with saline at minutes 10, 20 and 30 of the test ($p < 0.01$ in all cases). Moreover, MSEW High- and Low-PPI females treated with cocaine presented higher motor activity than those treated with saline at the 10th minute ($p < 0.05$ in two cases).

Motor sensitization data are presented in Fig. 2C and D. First, regarding habituation to the environment, the ANOVA revealed a main effect of the variable Rearing [$F(5,81) = 31.208$; $p < 0.0001$], and a significant effect of the two-way interaction Time x Pre-treatment [$F(5,81) = 3.631$; $p < 0.005$]. All animals decreased their spontaneous locomotor activity through time; however, females sensitized with cocaine showed higher motor activity than females treated with saline in the minutes 50 and 60 of the habituation phase (data not shown). Second, regarding cocaine sensitization, the four-way ANOVA showed a significant effect of the two-way interactions Rearing x Pre-treatment [$F(1,85) = 20.099$; $p < 0.0001$], Time x Pre-treatment [$F(2,84) = 56.061$; $p < 0.0001$], the three-way interactions Rearing x PPI x Pre-treatment [$F(1,85) = 8.559$; $p < 0.004$], Time x Rearing x Pre-treatment [$F(2,84) = 4.368$; $p < 0.016$], and the four-way interaction Time x Rearing x PPI x Pre-treatment [$F(2,84) = 3.726$; $p < 0.028$].

Post-hoc comparisons of the four-way interaction showed that cocaine pre-treatment caused a higher locomotor response to the challenge in all groups ($p < 0.01$) except in MSEW-Low PPI mice. In females pre-treated with vehicle, the locomotor response to cocaine was increased in SN High-PPI females at minutes 10, 20 and 30 ($p < 0.001$ in all cases); in SN Low-PPI females at minutes 10 and 20 ($p < 0.001$ in all cases); and in MSEW High-PPI and Low-PPI females at the 10th minute ($p < 0.05$ in all cases). In contrast, in females pre-treated with cocaine, at minutes 10, 20 and 30 of the test, MSEW Low-PPI females showed lower motor activity induced by challenge than SN Low-PPI and MSEW High-PPI ($p < 0.0001$ in all cases). At 20th minute of the test, vehicle MSEW Low-PPI females showed higher motor activity induced by challenge than their SN counterparts ($p < 0.012$). All these results revealed that MSEW Low-PPI females are less sensitive to the sensitization effects of cocaine pre-treatment.

The linear regression analysis showed a significant relationship between the PPI score and the hyperactivity induced by challenge in MSEW females pre-treated with cocaine [$F(1,27) = 6.232$; $p < 0.019$; $R = 0.440$ $R^2 = 0.193$] (see Fig. 2E).

3.2. Experiment 2. Effects of MSEW on CPP induced by cocaine

SN mice were classified as High- or Low-PPI animals using a two-cluster analysis [$F(1,20) = 45.829$; $p < 0.0001$]. Afterwards, MSEW females were categorized as High- or Low-PPI using the data obtained from the two-cluster analysis of K mean performed on the SN mice (High-PPI: from 9.39 to 33.70; Low-PPI: from -32.15 to 5.23). SN and MSEW females did not show significant differences in their PPI scores [$F(1,50) = 0.794$; $p = 0.377$] (SN Low-PPI: -5.8 n = 11; SN High-PPI: 20.91 n = 11; MSEW Low-PPI: -11.17 n = 10; MSEW High-PPI: 23.92 n = 19).

In the CPP induced by 1 mg/kg of cocaine, the ANOVA to analyse the time spent in the drug-paired compartment during pre- and post-conditioning revealed a significant main effect of the variable Days [$F(1,45) = 4.633$; $p < 0.037$] and the PPI x Days interaction [$F(1,45) = 5.782$; $p < 0.02$]. Post hoc comparisons for the interaction PPI x Days revealed that High-PPI females spent significantly more time in the drug-paired compartment in the Post-C than Low-PPI females ($p < 0.006$). The interaction PPI x Rearing x Days did not reach statistical significance, but the Tukey HSD test indicated that MSEW High-PPI females significantly increased the time spent in the drug-paired compartment in the Post-C compared to MSEW Low-PPI mice ($p < 0.01$); moreover, only SN and MSEW High-PPI females acquired CPP

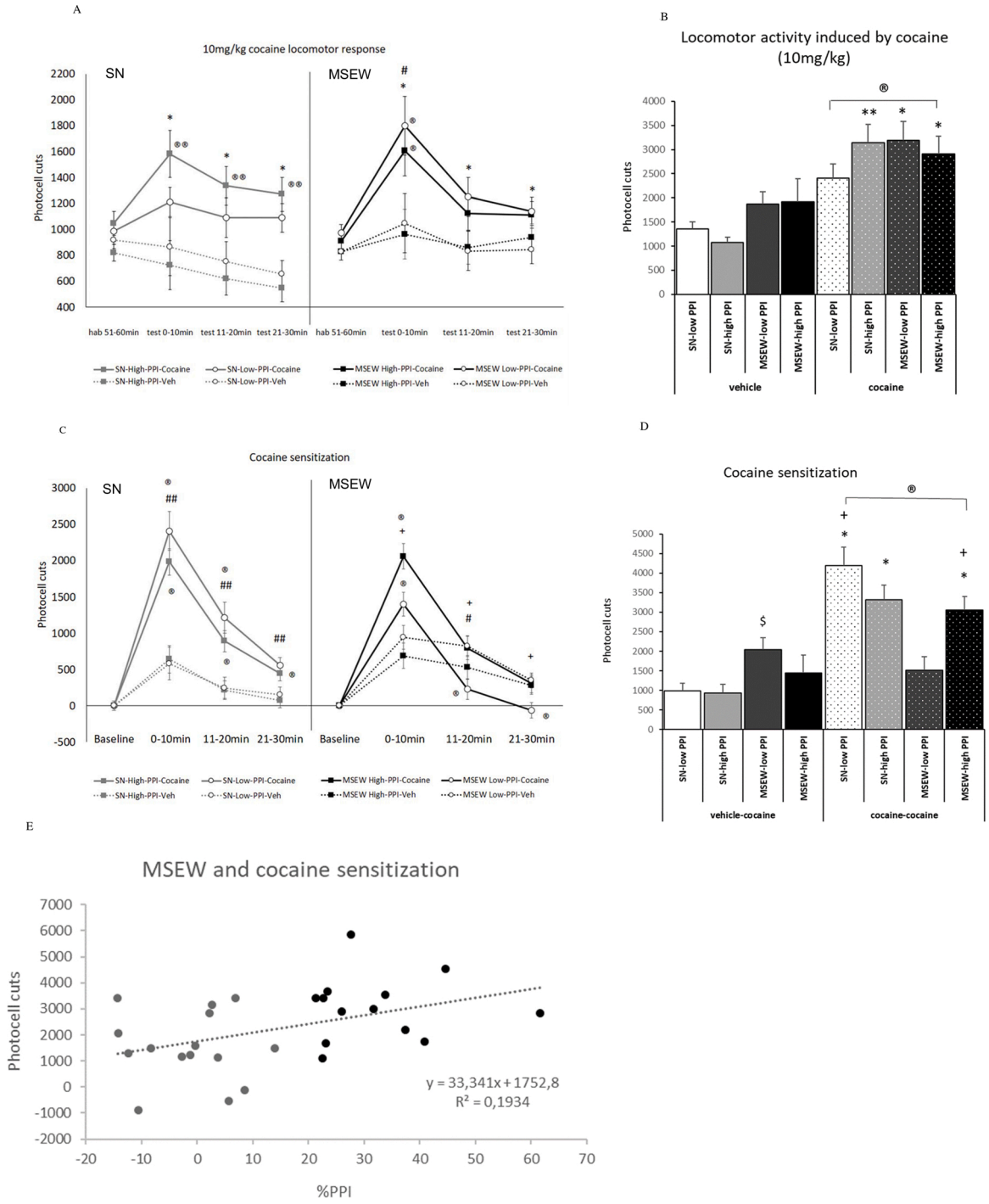

Fig. 2. Effect of MSEW on cocaine locomotor response and sensitization. **A**) Locomotor response induced by 10 mg/kg of cocaine or saline in SN and MSEW female mice categorized as High- and Low-PPI. Data presented as mean values ± S.E.M. during the 30 min period of locomotor activity in photocell cuts. #$p < 0.05$ with respect to SN (general Rearing effect). *$p < 0.001$ cocaine groups with respect to the habituation phase (Drug and Time interaction). ®®$p < 0.01$ and ®$p < 0.05$ cocaine vs. vehicle **B**) Total locomotor response induced by 10 mg/kg of acute cocaine or vehicle in SN and MSEW female mice categorized as High- and Low-PPI. ®$p < 0.05$ cocaine vs. vehicle (general Drug effect); *$p < 0.01$ vs. their vehicle counterparts. **C) Cocaine sensitization**. During the induction phase, the animals received a pre-treatment with saline or 25 mg/kg cocaine per day on three consecutive days. Five days later, all the animals received a challenge of cocaine 10 mg/kg. #$p < 0.01$; ##$p < 0.001$ SN vs MSEW corresponding group (Rearing effect); +$p < 0.01$ with respect to the corresponding Low-PPI group (PPI effect); ®$p < 0.01$ cocaine vs. vehicle pre-treated group (Pre-treatment effect, behavioural sensitization). **D**) Total locomotor activity induced by challenge (cocaine 10 mg/kg) in SN and MSEW females after pre-treatment with vehicle and cocaine. ®$p < 0.01$ cocaine vs. vehicle pre-treated group (Pre-treatment effect, behavioural sensitization); *$p < 0.01$ vs. their vehicle-cocaine counterparts; +$p < 0.01$ vs. MSEW-low PPI cocaine-cocaine. (SN Low-PPI n = 20; SN High-PPI n = 28; MSEW Low-PPI n = 25; MSEW High-PPI n = 28). **E**) The scatter plot for the relationship between the behavioural sensitization (total increase of locomotor activity induced by challenge) and the PPI percentage in MSEW females (grey circles for Low-PPI females and black circles for High-PPI females).

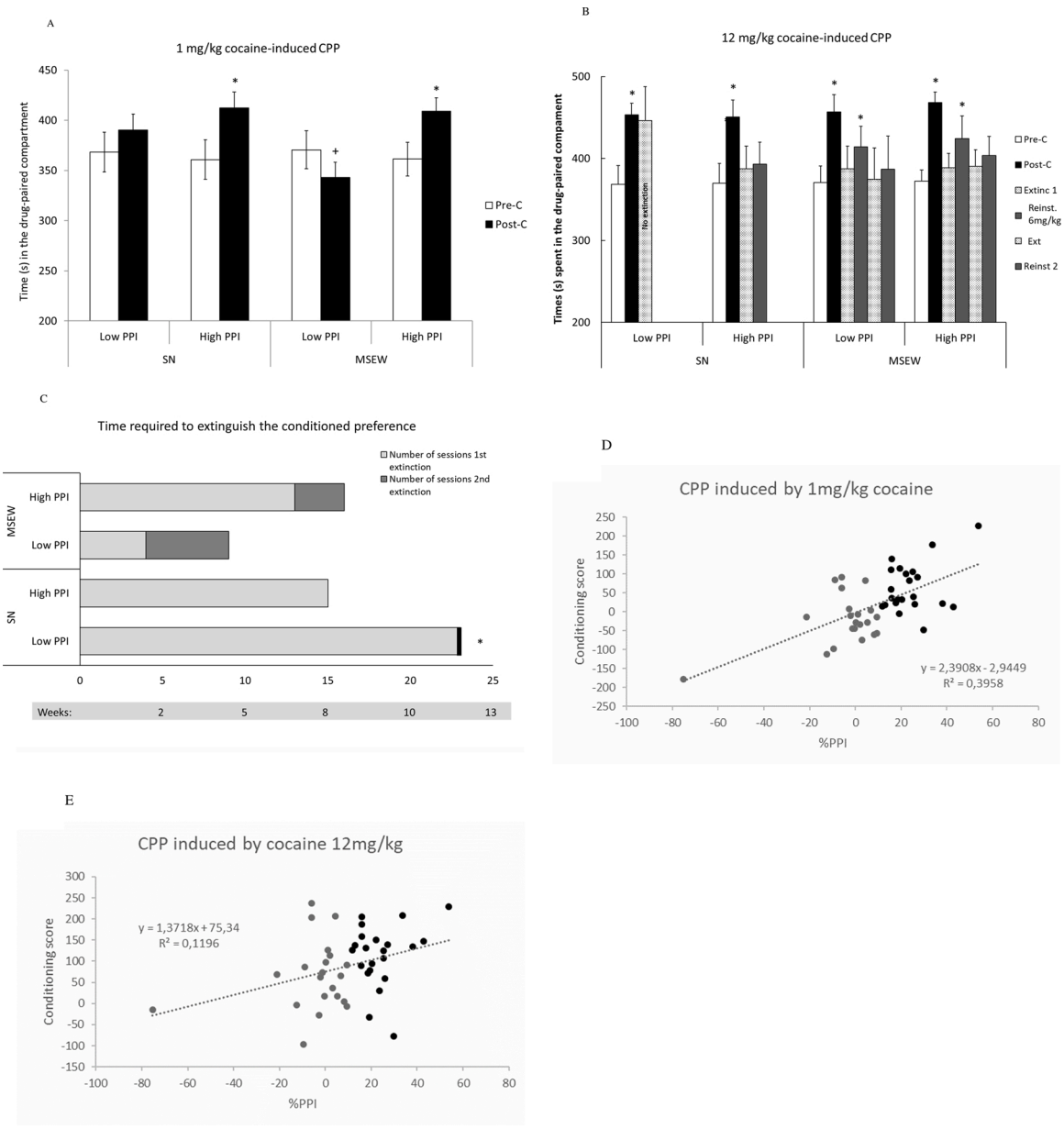

Fig. 3. Effect of MSEW on the cocaine-induced CPP. **A**) Conditioning place preference induced by 1 mg/kg of cocaine in SN and MSEW female mice categorized as High- and Low-PPI. Bars represent the mean (±SEM) time spent in the drug-paired compartment before conditioning sessions (white) and after conditioning sessions (black). *p < 0.05 vs. Pre-C, +p < 0.05 vs Post-C MSEW High-PPI. **B**) Conditioning place preference induced by 12 mg/kg of cocaine in SN and MSEW female mice categorized as High- or Low-PPI. Bars represent the mean (±SEM) time spent in the drug-paired compartment during pre-conditioning (white), during post-conditioning (black), the last extinction session (light grey) and reinstatement with 6 or 3 mg/kg of cocaine (dark grey). *p < 0.05 vs Pre-C or EXT. **C**) Number of sessions required for the preference induced by 12 mg/kg of cocaine to be extinguished after the Post-C test in SN and MSEW female mice categorized as High- or Low-PPI. Kaplan–Meier test: *p < 0.05 with respect to the rest of the groups (SN Low-PPI n = 11; SN High-PPI n = 11; MSEW Low-PPI n = 10; MSEW High-PPI n = 19). **D**) The scatter plot for the relationship between the conditioning score of CPP induced by 1 mg/kg of cocaine and the percentage of PPI (grey circles for Low-PPI females and black circles for High-PPI females). **E**) The scatter plot for the relationship between the conditioning score of CPP induced by 12 mg/kg of cocaine and the percentage of PPI (grey circles for Low-PPI females and black circles for High-PPI females).

(Fig. 3A).

In the CPP induced by 12 mg/kg of cocaine, the ANOVA revealed a significant main effect of the variable Days [$F(1,48) = 50.730$; $p < 0.0001$], showing that all animals acquired CPP (Fig. 3B). To evaluate the extinction-reinstatement process individually, several *t*-test comparisons were performed, which showed that only the MSEW groups reinstated the preference with a priming dose of 6 mg/kg of cocaine (MSEW Low-PPI: $t(10)=-2.791$ $p < 0.019$; MSEW High-PPI: $t(16)=-2.137$ $p < 0.048$). The SN High-PPI group did not reinstate the conditioned preference with 6 mg/kg cocaine and the SN Low-PPI group never extinguished the preference. The number of sessions required for the preference to be extinguished after conditioning and after reinstatement induced by cocaine-priming are presented in Fig. 3C. Kaplan-Meyer analysis of the data obtained after post-conditioning revealed that the SN Low-PPI group took longer than the other groups to extinguish the preference, since they did not reach extinction (vs. SN High-PPI: $\chi2 = 4.208$, $p < 0.04$; and vs. MSEW Low-PPI: $\chi2 = 4.668$; $p < 0.031$).

The females that showed CPP induced by cocaine in each group were: MSEW low-PPI 18,2% with 1 mg/kg and 54,5% with 12 mg/kg; MSEW high-PPI 38,5% with 1 mg/kg and 92,3% with 12 mg/kg; SN low-PPI 22,2% with 1 mg/kg and 66,7% with 12 mg/kg; SN high-PPI 50 % with 1 mg/kg and 70 % with 12 mg/kg.

The linear regression analysis showed a significant relationship between the PPI score and the time in the drug-paired compartment in the Post-C in all females [$F(1,42) = 16.156$; $p < 0.001$; $R = 0.532$ $R^2 = 0.283$], and in MSEW females [$F(1,23) = 12.189$; $p < 0.002$; $R = 0.597$ $R^2 = 0.357$] in CPP induced by cocaine 1 mg/kg. Fig. 3D and E shows the linear association between PPI score and preference score.

3.3. Experiment 3. Effects of PPI but not MSEW on operant cocaine self-administration

SN mice were classified as High- or Low-PPI animals using a two-cluster analysis [$F(1,37) = 64.289$; $p < 0.0001$]. Afterwards, MSEW females were categorized as high or low PPI using the data obtained from the two-cluster analysis of K mean performed on the SN mice (High-PPI: from 1.03 to 24.86; Low-PPI: from -41.85 to -1.7). SN and MSEW females did not show significant differences in their PPI scores [$F(1,65) = 1.483$; $p = 0.228$] (SN Low-PPI: -16.08 n = 15; SN High-PPI: 12.04 n = 21; MSEW Low-PPI: -8.85 n = 15; MSEW High-PPI: 9.84 n = 18). Only animals who survived the surgery were included in the statistical analysis (SN = 31; MSEW = 31).

The analysis of the infusions in the MSEW and SN groups in both High- and Low-PPI mice revealed different responses in the females depending on the rearing conditions and the PPI categorization (Fig. 4A and B). The four-way ANOVA analysis (Day × Hole × PPI × Rearing) showed significant main effects of Day [$F(9,522) = 5.39$, $p < 0.001$], Hole [$F(1,58) = 59.28$, $p < 0.001$], as well as a significant interaction Day × Hole [$F(9,522) = 9.11$, $p < 0.001$] and Hole × Days × Rearing × PPI [$F(9,522)=2.210$, $p < 0.05$]. The post-hoc pairwise comparison Hole × Days × Rearing × PPI evidenced that SN Low-PPI females achieved a higher number of infusions than MSEW Low-PPI on days 5, 6, 7 and 9 ($p < 0.05$ in all cases). The post-hoc analysis also revealed that the SN Low-PPI group achieved a higher number of infusions than the SN High-PPI group but only on day 6 ($p < 0.05$; Fig. 4A).

A further detailed analysis regarding the rearing conditions showed that the SN High-PPI group discriminated between the active and inactive holes on days 2, 3, 4, 6, 7, 8, 9 and 10 ($p < 0.05$ in all cases; Fig. 4A) whereas the SN Low-PPI group discriminated between the holes on days 2, 4, 5, 6, 7, 8, 9 and 10 ($p < 0.05$ in all cases; Fig. 4A). However, MSEW female mice had a different time-course in the discrimination between holes. In this case, MSEW High-PPI mice were able to

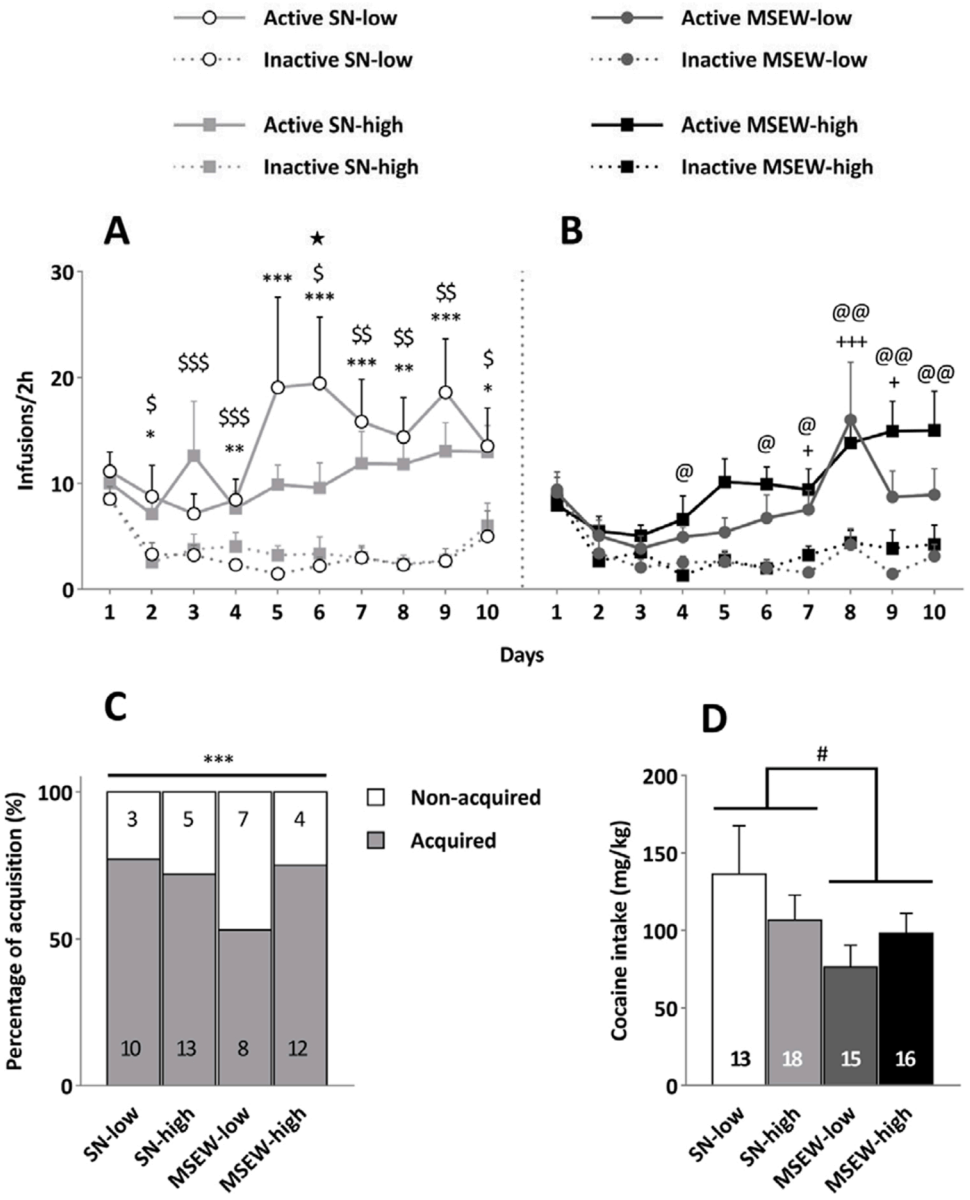

Fig. 4. Effect of MSEW on the cocaine SA behaviour during the acquisition phase. A) Mean of infusions in the active/inactive hole during the 10 days of the cocaine self-administration in SN-low (n = 13) and SN-high (n = 18) female mice. **B)** Mean of infusions in the active/inactive hole during the 10 days of the cocaine self-administration in MSEW-low (n = 15) and MSEW-high (n = 16) female mice. Discrimination between active/inactive holes in SN-low (*p < 0.05, **p < 0.01, ***p < 0.001), SN-high ($p < 0.05, $$p < 0.01, $$$p < 0.001), MSEW-low (+p < 0.05, +++p < 0.001), MSEW-high (@p < 0.05, @@p < 0.01) group; ★ p < 0.05 active infusions between SN-low and SN-high. **C)** Percentage of animals reaching acquisition criteria (***p < 0.001) Chi square test. **D)** Mean of total cocaine intake (mg cocaine mg/kg mice) along the acquisition phase (#p = 0.057). Data are expressed as mean ± SEM.

discriminate between the active and inactive holes on days 4, 6, 7, 8, 9 and 10 ($p < 0.05$ in all cases; Fig. 4B), whereas MSEW Low-PPI mice differentiated between holes at a later time, only on days 7, 8 and 9 ($p < 0.05$ in all cases; Fig. 4B).

Considering the criteria of acquisition, the percentage of mice that acquired the cocaine self-administration behaviour was 77 % for SN Low-PPI, 72 % for SN High-PPI, 53 % for MSEW Low-PPI and 75 % for MSEW High-PPI groups (Fig. 4C). A Chi square test showed a significant difference in percentage of acquisition between the groups ($p < 0.001$). For the total cocaine intake, the two-way ANOVA analysis (PPI × Rearing) showed a tendency for Rearing conditions [$F(1,58) = 3.766$, $p = 0.057$], and PPI x Rearing [$F(1,58) = 2.844$, $p = 0.09$]. Our data revealed that SN females consume more cocaine than MSEW females (Fig. 4D), and if the post-hoc analysis are tested in the tendency for PPI x Rearing, SN Low-PPI females achieved a higher number of infusions than MSEW Low-PPI mice ($p < 0.01$).

3.4. Behavioral profile

3.4.1. Splash test: MSEW increased the anhedonia-like behaviour in Low-PPI females

In the splash test, the ANOVA for total grooming showed a significant effect of the interaction Rearing x PPI [$F(1,39) = 6.445$; $p < 0.015$]. The post-hoc pairwise comparison revealed a lower percentage of self-grooming time in MSEW Low-PPI females than SN Low-PPI ($p < 0.05$) mice, and a higher percentage of self-grooming time in SN Low-PPI

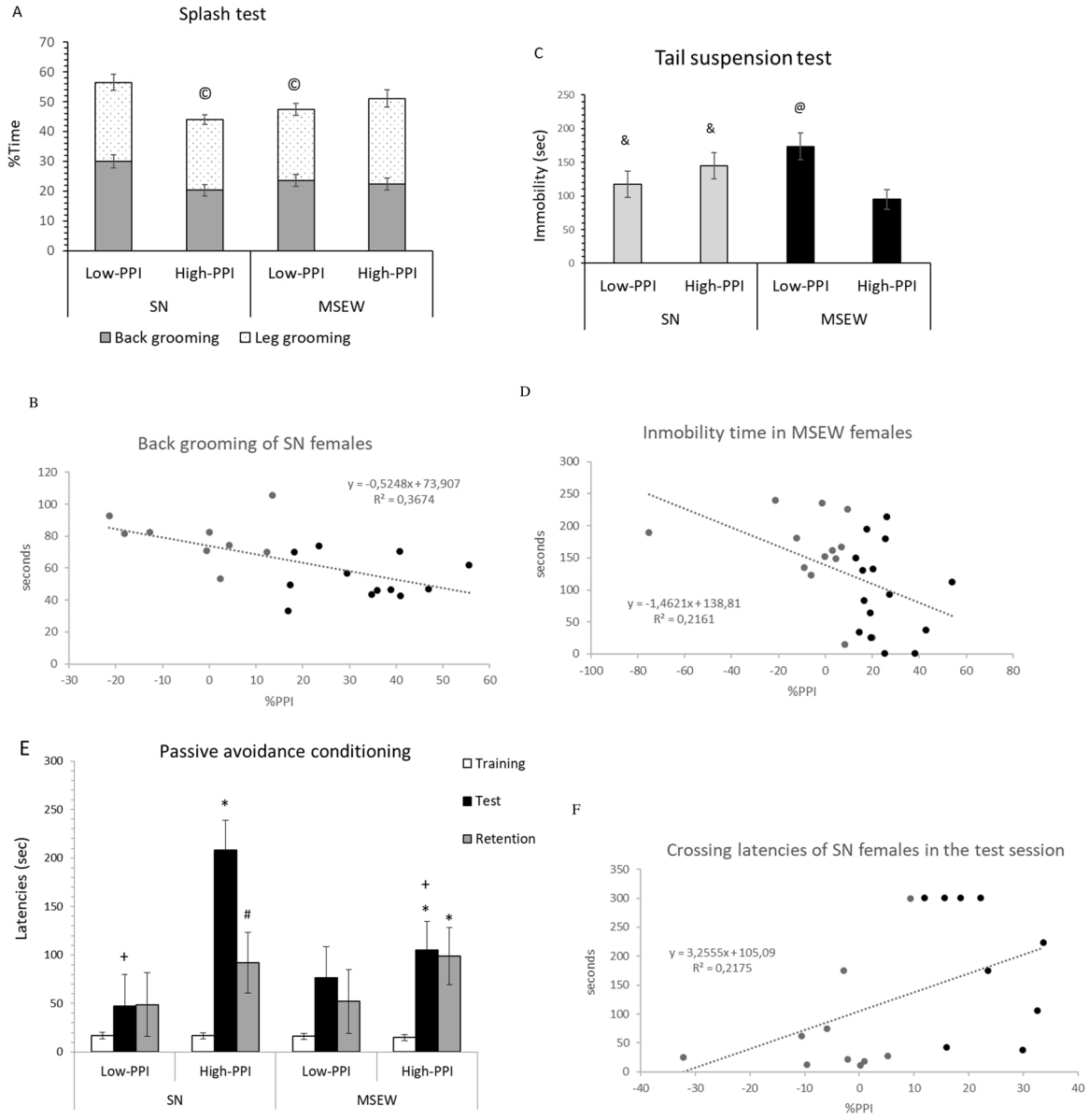

Fig. 5. Effect of MSEW on depressive-like behaviour and memory. **A**) Percentage of time in leg and back grooming in the Splash test. ©$p < 0.01$ vs. SN Low-PPI females (SN Low-PPI n = 9; SN High-PPI n = 12; MSEW Low-PPI n = 11; MSEW High-PPI n = 11). **B**) The scatter plot for the relationship between the time (sec) in back grooming and the PPI percentage (grey circles for Low-PPI females and black circles for High-PPI females). **C**) Time of immobility in the Tail Suspension Test. &$p < 0.05$ vs. MSEW; @$p < 0.01$ vs. MSEW High-PPI (SN Low-PPI n = 10; SN High-PPI n = 10; MSEW Low-PPI n = 10; MSEW High-PPI n = 18). **D**) The scatter plot for the relationship between the time (sec) of immobility and the PPI percentage in MSEW females (grey circles for Low-PPI females and black circles for High-PPI females). **E**) Crossing latency to the dark compartment associated with punishment in the Passive Avoidance Conditioning, 24 h and 7 days after training session. *$p < 0.001$ vs. Training; +$p < 0.001$ vs. SN High-PPI females; #$p < 0.001$ vs. test session. (SN Low-PPI n = 9; SN High-PPI n = 10; MSEW Low-PPI n = 9; MSEW High-PPI n = 11). Data are presented as mean values ± S.E.M. **F**) The scatter plot for the relationship between the crossing latencies (sec) of SN females in the test session and the PPI percentage in MSEW females (grey circles for Low-PPI females and black circles for High-PPI females).

females than SN High-PPI ($p < 0.009$) (see Fig. 5A). The ANOVA for back grooming showed a significant effect of the PPI variable [$F(1,39) = 7.402$; $p < 0.01$], with High-PPI females displaying less back grooming than Low-PPI females; and a significant effect of the interaction Rearing x PPI [$F(1,39) = 4.449$; $p < 0.041$], with MSEW Low-PPI females showing less back grooming than SN Low-PPI ($p < 0.035$) mice (see Fig. 5A). However, the ANOVA for leg grooming did not reveal any significant effect.

The linear regression analysis showed a significant relationship between the PPI score and the back grooming of SN females [$F(1,20) = 11.033$; $p < 0.004$; $R = 0.606$ $R^2 = 0.367$] (see Fig. 5B).

3.4.2. Tail suspension test: MSEW increased the despair-like behaviour in low-PPI females

In the tail suspension test, the ANOVA for immobility time showed a significant effect of the interaction Rearing x PPI [$F(1,44) = 8.105$; $p < 0.007$]. The post-hoc pairwise comparison revealed that MSEW increased the immobility time in Low-PPI females ($p < 0.05$), but it decreased the immobility time in High-PPI females ($p < 0.048$); and MSEW-Low PPI females presented a longer time in immobility than MSEW-High PPI ($p < 0.003$) (see Fig. 5C).

The linear regression analysis showed a significant relationship between the PPI score and the immobility time in MSEW females [$F(1,21) = 7.052$; $p < 0.015$; $R = 0.511$ $R^2 = 0.261$] (see Fig. 5D).

3.4.3. Passive avoidance task: low-PPI females displayed lower retention of an aversive event

The ANOVA for crossing latencies in the inhibitory avoidance conditioning task showed a significant effect of the Sessions variable [$F(2,34) = 16.812$; $p < 0.0001$] and the PPI variable [$F(1,35) = 6.237$; $p < 0.017$], displaying Low-PPI females as having lower crossing latencies than High-PPI females. The ANOVA also revealed a significant effect of the interactions PPI x Sessions [$F(2,34) = 4.434$; $p < 0.019$] and Rearing x PPI x Sessions [$F(2,34) = 3.199$; $p < 0.05$]. The post-hoc pairwise comparison Rearing x PPI x Sessions demonstrated that SN Low-PPI females showed lower latencies than SN High-PPI mice in the test session ($p < 0.001$) and that MSEW significantly decreased the crossing latencies of High-PPI mice in the test session in comparison with SN High-PPI female ($p < 0.021$). Moreover, SN High-PPI females significantly increased the latencies in the test session vs. the training session ($p < 0.0001$); but they decreased their latencies in the retention session vs. the test session ($p < 0.001$). However, MSEW High-PPI females significantly increased the latencies in the test session vs. the training session ($p < 0.013$), and the retention session vs. the training session ($p < 0.022$) (see Fig. 5E).

The linear regression analysis showed a significant relationship between the PPI score and the crossing latencies of SN females in the test session [$F(1,18) = 4.727$; $p < 0.044$; $R = 0.466$ $R^2 = 0.218$] (see Fig. 5F).

4. Discussion

The main goal of the present study was to evaluate the predictive ability of the PPI paradigm to identify females with a greater vulnerability to the long-term consequences of an early stress (MSEW) on the locomotor, conditioned rewarding and motivational effects of cocaine. Our results revealed that female mice exposed to MSEW presented the negative consequences derived from stress, particularly those with a low PPI profile. Throughout the variety of results presented in this study, we confirm that MSEW aggravates the behavioural and emotional status in Low-PPI mice, which would correspond to the part of the population that presents psychiatric pathologies.

Our results revealed that Low-PPI animals exposed to MSEW showed an increased response to the acute locomotor effects of cocaine, but did not show sensitization induced by a protocol used in previous studies [34,39]. Regarding CPP induced by a subthreshold dose and an effective dose of cocaine according to previously published work by our research group [33,39,41,49,50], these Low-PPI females seemed to be less sensitive to the conditioned rewarding effects of cocaine. The groups with a low PPI did not acquire CPP with the subthreshold dose of cocaine (1 mg/kg). However, when administering an effective dose (12 mg/kg) in the CPP, MSEW Low-PPI animals exhibited reinstatement with half the previous dose (6 mg/kg), a fact that did not occur in non-stressed mice. In the same line, MSEW mice (high and low PPI) exhibited a decrease in cocaine SA in comparison to their SN corresponding groups, but cocaine intake was especially impaired in those presenting a low PPI, which can be explained in part by a lower acquisition of SA. Finally, these results are supported by the emotional profile, as MSEW Low-PPI females showed an increased anhedonia-like behaviour, proven in the splash and tail suspension tests.

Regarding the locomotor response to cocaine, an early stress such as MSEW induced a slight increase in the acute locomotor effects of cocaine in Low-PPI females only; nonetheless, when they were pre-treated with cocaine, sensitization was not manifested in Low-PPI females, as opposed to SN females. An attenuated sensitization in MSEW mice has also been observed in a previous study with adolescent male mice [23], though using a different protocol to ours. In the present study, the increased hyperactivity induced by an acute dose of cocaine and the weakened motor sensitization induced by cocaine pre-treatment was mainly observed in animals exposed to MSEW with a low PPI profile. On the other hand, their non-stressed counterparts (SN Low-PPI) displayed a lower sensitivity to the acute motor effects of cocaine but an increased locomotor sensitization. Thus, a linear relation was observed between PPI and the hyperactivity induced by cocaine in sensitized females exposed to MSEW. These results are in line with other studies that suggest an increased vulnerability in Low-PPI mice (males and females), with an increased amphetamine [51] and cocaine sensitization [34]. Thus, in the present work we highlight that adding the social stressor MSEW completely changes both the acute locomotor and sensitization responses to cocaine, supporting previous results [23,24,52].

On the other hand, regarding the effects of MSEW on cocaine-related environmental cues, it was observed that all MSEW animals, independently of their PPI profile, reinstated the conditioned preference in the CPP with a priming dose of cocaine (6 mg/kg) while their non-stressed counterparts did not. Going further and focusing on the low-PPI profile, non-stressed females displayed the preference for a longer period of time, as they did not reach extinction. This confirms what a previous study reported in males, where low-PPI male mice persevered in their conditioned response, lengthening the extinction process [33]. In addition, Low-PPI females have been shown to exhibit increased cocaine-primed reinstatements with lower doses than their high-PPI counterparts. In the present work, this persistence can be explained by the previous exposure to the 1 mg/kg cocaine dose for four days, which could be acting as a priming or sensitization for the subsequent 12 mg/kg dose. This would be in line with the results obtained in the cocaine locomotor sensitization test, as SN Low-PPI females exhibited a higher sensitization than their SN High-PPI counterparts.

Furthermore, it has been confirmed that a 1 mg/kg dose is not enough to induce CPP in the non-stressed group (SN-Low PPI), observing a linear relation between the PPI sensitivity and the conditional reinforcing effects of cocaine. Previous studies have reported this dose as subthreshold because only animals with high sensitivity to drug rewarding effects acquire CPP [15,49,50]. Moreover, in a previous study it was demonstrated that Low-PPI mice do not acquire CPP with 1 mg/kg nor 6 mg/kg [33], which confirms again that Low-PPI mice do not experience the rewarding effects of cocaine at low doses, pointing to an impaired dopaminergic system.

Going further and considering the consequences of MSEW on the motivational attributes of cocaine reward by employing the SA paradigm, it appeared that MSEW only caused a trend to reduce the total cocaine intake in females, stronger in Low-PPI females than in High-PPI females. This result is in line with a previous study, where mice exposed to MSEW did not change their total cocaine intake [13]. However, that

study showed that being exposed to MSEW also enhanced the percentage of cocaine acquisition and cocaine-seeking behaviour, effects that have not been observed in the present work, where Low-PPI MSEW females exhibited a decreased acquisition with respect to the rest of the groups.

With regards to the performance of different PPI profiles in the SA, several interesting effects unseen in previous studies appear. Non-stressed Low-PPI females achieved a higher number of infusions than their high-PPI counterparts in specific days, confirming the lower sensitivity to the reinforcing effects of cocaine in this group. This supports the CPP results and previous studies, where the PPI sensitivity of mice can predict the response to dopaminergic agonists [33,34,51,53–55]. These differences between low- and high-PPI mice could be explained in part by changes in D1 and D2 dopamine receptors in the striatum, where a previous study showed that Low-PPI mice, particularly females, showed a higher D2 receptor expression [33]. This suggests the need of Low-PPI mice for larger doses of cocaine to acquire the CPP and their higher number of infusions in the SA, since an enhance in D2 receptor levels in the striatum has been related to a lack of pleasant effects of the psychostimulants [56].

Finally, this lack of sensitivity in Low-PPI females is intimately related to the results obtained in the anhedonia and despair tests. In this study, non-stressed Low-PPI mice exhibited the grooming behaviour for a longer time than the rest of the groups, specifically back grooming, which can be interpreted as a greater motivation for sucrose [40,57] but also related to rodent models of psychosis, since grooming behaviour is also modulated by the dopaminergic system [58]. In support of this, other studies have reported that, after being exposed to a restraint stress, mice showed a decreased time of grooming together with changes in dopaminergic activity, increasing in the mesolimbic area and decreasing in the prefrontal cortex [59]. The present work, which employed social stress instead of physical stress, confirms that exposure to MSEW increases anhedonia-like behaviours, but only in the most vulnerable group: the Low-PPI females. Being exposed to MSEW reduced their time of total and back grooming in comparison to their high-PPI counterparts.

Furthermore, results regarding the tail suspension test indicate that MSEW increases immobility time in Low-PPI mice but decreases it in High-PPI mice, observing a linear relation between the PPI sensitivity and the immobility time in stressed females. The time of immobility in this test is considered to be a measure of anhedonia in mice [43,60,61], confirming again the Low-PPI mice vulnerability to the consequences of stress. Previous studies with maternal separation as an early stressful environment indicate several neurochemical, cognitive and emotional alterations [23,24,52], suggesting an increased risk of neurological disorders and drug abuse in adulthood [19,62]. Ten years ago, Miller et al. [63] studied the effects of MSEW in females, reporting an enhanced microglial activation and neuroplasticity changes related to the development of depressive states, which would support the present anhedonia results.

The passive avoidance conditioned task induces learning through punishment, provoking a high emotional state in the subject [44]. The results of the present study demonstrate that females with lower PPI retain for a shorter time the memory of the negative stimulus or punishment than High-PPI females. Additionally, being exposed to MSEW also reduces the retention of castigation, more so in High-PPI females than Low-PPI subjects because the SN litter showed a very poor memory of the punishment.

The results of the present study, together with our previous work [33,34], endorse the utility of the PPI paradigm as an indicator of animals with a higher vulnerability to the effects of cocaine. Notwithstanding, a possible limitation to this paradigm is that it is not possible to determine a PPI absolute value to establish which animals have a low PPI, this being determined instead by a relative value as compared to the rest of the group evaluated. Nevertheless, it should be noted that a low PPI is not a symptom, but a risk factor to develop a cocaine use disorder.

5. Conclusions

We can affirm that MSEW has greater consequences on low-PPI females than in their high-PPI counterparts. Being exposed to MSEW altered the locomotor response to cocaine in low-PPI females and partially some of the cocaine-rewarding effects in the CPP and SA paradigms. In addition, the same group exhibited an increased anhedonia- and despair-like behaviours compared to their high-PPI counterparts. These results point to the role of stress as a modulating factor, which is a highly common circumstance in humans and animals alike. Low-PPI females displayed a poor coping response, while high-PPI females were more resilient to the negative consequences of stress. Future studies should not only focus on PPI's ability to discriminate vulnerable subjects, but also on finding successful resilience strategies that can protect them from the deleterious consequences of stress.

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Ethical statement

The experimental protocol has been approved by an Institutional Review Committee for the use of animal subjects. All procedures involving mice and their care were conducted in accordance with national (BOE-2013-1337) and EU (Directive 2010-63EU) guidelines regulating animal research and were approved by the ethics committee of the Pompeu Fabra University (CEEA-PRBB) and the University of Valencia. All the efforts were made to minimize animal suffering and to reduce the number of animal used.

Author contributions

M. Carmen Arenas: conceptualization, methodology, validation, formal analysis, writing, visualization, supervision, project administration and funding acquisition; Adriana Castro-Zavala: methodology, formal analysis, investigation, writing and visualization; Ana Martín-Sánchez: investigation, writing and visualization; María Carmen Blanco-Gandía: investigation, formal analysis, writing and visualization; José Miñarro: funding acquisition and writing-review and editing; Olga Valverde: conceptualization, methodology, formal analysis, funding acquisition, writing-review and editing; Carmen Manzanedo: conceptualization, methodology, validation, investigation, formal analysis, writing-review and editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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