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Research report

Prenatal stress increases adult vulnerability to cocaine reward without affecting pubertal anxiety or novelty response

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ABSTRACT

Prenatal stress (PS) induces long-lasting molecular alterations in brain circuits of the offspring and increases the propensity to develop neuropsychiatric diseases during adulthood, including mood disorders and drug addiction. A major goal of this study was to assess the impact of PS on pubertal behaviour and adult vulnerability to cocaine-induced conditioning place preference (CPP). We therefore evaluated pubertal novelty response and anxiety-like behaviour in control (C) and PS rats, and then, we examined cocaine-induced CPP in those animals during adulthood. We found no differences between C and PS groups on pubertal behaviour, however, only PS rats showed a significant cocaine-induced CPP. To further analyze our results, we classified cocaine-treated rats regarding their CPP score in Low CPP or High CPP and we then analysed their pubertal behaviour. We found different relations of anxiety-like behaviour to cocaine reward as a function of PS exposure: for C group, High CPP and Low CPP had shown similar levels of anxiety-like behaviour at puberty; on the contrary, for PS group, High CPP had shown lower anxiety-like behaviour than Low CPP rats. This study underscores the importance of considering prenatal exposure to stress when analysing the relationship between anxiety and cocaine vulnerability. Moreover, the evaluation of behavioural traits at puberty opens the possibility of early intervention and will allow the development of specific prevention strategies to avoid the devastating consequences of drug addiction later in life.

1. Introduction

Substance use disorder (SUD) is a major health, social and economic problem worldwide, defined in the Diagnostic and Statistical Manual of Mental Disorders version V (DSM-V) as a: "…cluster of cognitive, behavioural, and physiological symptoms indicating that the individual continues using the substance despite significant substance related problems" [1]. An aspect that has lately drawn considerable attention is the fact that only a small percentage of people which experiment with potentially addictive drugs develop a SUD [2,3], even for a very addictive drug like cocaine [4,5]. Despite the fact that the aetiology of SUD remains poorly understood, it is largely accepted that individual susceptibility to develop this devastating disease is related to genetic and environmental risk factors common to multiple substances [6–13]. In rodent models, prenatal stress (PS) has also been proposed as an important player in the development of SUD (reviewed by [14–16]), triggered by the exposure to different drugs of abuse, including cocaine [17–19].

In view of the significant variability in drug reward susceptibility across individuals, researchers had investigated behavioural traits that predispose them to develop SUD following drug exposure. In this sense, anxious behaviour and novelty response have previously been associated with cocaine vulnerability, although some inconsistencies are present across rodent studies [20–28]. Importantly, previous reports did not investigate the influence of PS on those associations, although individual differences to cocaine reward are likely due to ontogenetic changes in brain regions related with the reward system [29]. Moreover, it is interesting to notice that in previous studies both anxiety trait/novelty response and cocaine effects were assessed during adulthood. Given that puberty is a period of higher vulnerability to risk-taking, novelty seeking, anxiety and lack of self-regulation [30–33], we examined the influence of PS on pubertal anxiety-like behaviour/novelty response and adult cocaine-induced reward, with the aim to find any relationship between those pubertal behavioural traits and adult vulnerability to cocaine effects as a function of PS exposure.

Abbreviations: C, control (non-prenatally stressed); COC, cocaine; CPP, conditioning place preference; EPM, elevated plus maze; OF, open field; PS, prenatal stress; SAL, saline; SAP, stretch attend posture; SUD, substance use disorder

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2. Material and methods

2.1. Animals

Seventeen virgin female Wistar rats weighing 250–280 g and sexually experienced Wistar male rats weighing 400–450 g were obtained from outbred rats belonging to the animal facility of the School of Pharmacy and Biochemistry, University of Buenos Aires. A maximum of four rats were housed per cage with *ad libitum* access to standard rat chow (Asociación de Cooperativas Argentinas– Buenos Aires, Argentina) and water. A constant light/dark cycle (12:12), with lights on at 07:00 h and off at 19:00 h, and a room temperature of 23–25 °C were maintained. Females were individually mated with a male in a mating cage. The day on which vaginal plug was found was designated as the first day of pregnancy. All procedures were in agreement with the standards for the care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and were approved by the Institutional Animal Care and Use Committee (CICUAL Res (CD) N° 2235/2016, School of Medicine, University of Buenos Aires). Care was taken to minimize the number of animals used and their suffering.

2.2. Drugs

For place conditioning experiments, a dose of 20 mg/kg cocaine hydrochloride (Verardo Laboratory, Buenos Aires, Argentina) was dissolved in saline solution (NaCl 0.9%) and administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. An equal volume of saline solution was injected to control group. 1/2 inch 30 G needles were used to minimize possible discomfort associated with injections. Indicated dose is based on the molecular weight of the free-base.

2.3. Prenatal stress

Pregnant dams were randomly assigned to either the prenatal stress (PS) or control (C) group and were individually housed with *ad libitum* access to standard rat chow and water. C rats (n = 8) were left undisturbed in the home cage, while PS dams (n = 9) were subjected to a restraint stress procedure, as previously described [34,35]. Briefly, pregnant females were individually placed into a transparent plastic restrainer fitted closely to body size for three 45-min periods per day (09:00, 12:00 and 16:00 h) between days 14 and 21 of pregnancy. The restrainer had ventilation holes, and dimensions appropriate for a pregnant rat of 350 g: internal diameter 64 mm, and an adjustable length of 149–208 mm. This type of stressor was chosen because it has an indirect influence on the foetuses via a direct stress on the mother [36,37]. The sessions were performed in a lit environment. No other subjects were present in the experimental room during the stress exposure. At the end of the stress session, rats were returned to the animal housing room and were then individually housed with free access to food and water. Fig. 1 shows a schematic diagram of the experimental design. On the day of parturition, litter characteristics were recorded and litters were culled to 10 pups, maintaining similar number of males and females, when possible. Weaning was performed at postnatal day 21. For the present study, we used male offspring, which were housed in separated cages with no more than 5 rats per cage, and in standard housing conditions. To avoid litter effects, a maximum of two pups from each litter were tested for each experimental group.

2.4. Behavioural measurements of the offspring

Anxiety-like behaviour and novelty response were evaluated during puberty (P34-P35) and then the animals were left undisturbed until adulthood (P90), when they were trained in a CPP protocol. Animals were handled during 2–3 min once a day for 4 days prior to pubertal and adult behavioural experiments to avoid acute stress. Behavioural tests took place during the morning and the animals were habituated to the behavioural room for 1 h. The apparatuses were cleaned with 70% ethanol and completely dried between each animal testing. All experiments were videotaped with a camera placed 1 m above the apparatuses. Except for the EPM task, experimenters were absent from the room during behaviour recording. Then, the videos were analysed using Kinovea (http://www.kinovea.org) or Solomon Coder Software (http://www.solomoncoder.com) by a researcher blind to experimental groups.

2.4.1. Elevated plus maze (EPM) task during puberty

Anxiety-like behaviour was assessed at postnatal day 34 using a standard elevated plus maze described elsewhere [38,39]. Briefly, the apparatus consisted of two open (45 cm length × 10 cm width) and two closed arms (45 cm length × 10 cm width × 50 cm height) opposite to each other inter-connected by a central platform (10 × 10 cm) and was elevated 65 cm over the floor. Each animal was placed in the centre, facing an open arm, and left in the maze during 5 min [25,40]. Time spent in the open arms and the number of entries to the open arms were used as indices of anxiety, whereas the number of entries to the closed arms was used as an index of general locomotor activity (an entry was defined as an entry of all four limbs into an arm of the maze) [41–43]. Number of head dipping (protruding the head over the ledge of an open arm and down towards the floor, which can occur while the animal's body is in the central square or the open arms) and number of stretching attend postures (SAPs, the rat stretches forward and retracts to original position with the hind paws fixed, which can occur in any part of the maze) were also quantified.

2.4.2. Open field (OF) task during puberty

Locomotor response to novelty was assessed 24 h following EPM test, at postnatal day 35 in an open field black arena (65 cm length × 65 cm width × 47 cm height). After the acclimatization period to the behavioural room, each animal was placed individually in the centre of the open field arena during 20 min and then returned to its home cage. Locomotor activity (total distance travelled in cm), number of rearing (rising on the hind limbs both touching and not touching a wall surface) and grooming (friction of any part of the body with the paws and/or the mouth) behaviours were quantified during that time.

2.4.3. Conditioning place preference (CPP) task during adulthood

Cocaine-induced CPP was assessed between postnatal days 90–100 in a three-compartment box (Fig. 1). CPP boxes exhibited two similar compartments (30 length × 25 width × 30 cm height), one black and the other white, separated by a small grey compartment (12 × 25 × 30 cm) with sliding doors. The two large compartments had different visual and tactile cues: one was totally black with a bar-grid floor, whereas the other was totally white with a wire mesh floor. The conditioning box used in this study is considered biased because animals showed a significant preference for the black compartment over the white one prior to conditioning [44,45]. The position of the rats was recorded through a video camera during the whole experiment and the time spent in the conditioning compartments and central chamber was measured by a researcher blind to treatment condition. The white compartment was fitted with a pair of lines on the floor (4 cm from each end of the chamber), and horizontal locomotor activity was measured by a researcher blind to experimental groups, using the Solomon Coder Software and counting only when lines at both ends of the cage were interrupted consecutively [46]. The place conditioning procedure was conducted on the diurnal phase of the light/dark cycle [47] and consisted of three phases: Habituation and Pretest (days 1 and 2), Conditioning (days 3–10), and CPP Test (day 11).

2.4.3.1. Habituation and pretest. The first and second days of CPP procedure, animals were placed in the CPP box with doors opened, and allowed to roam freely the three compartments for 15 min. The first

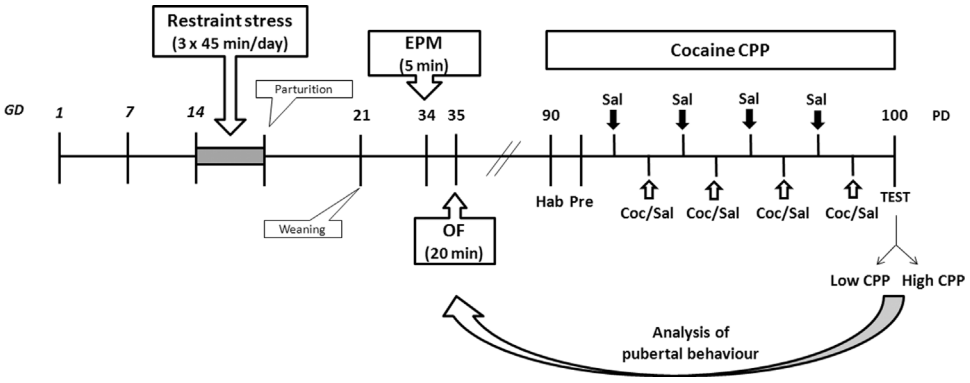

Fig. 1. Schematic diagram showing the experimental design. During the last week of gestation, PS dams were exposed to the restraint stress protocol while control dams were left undisturbed. Pubertal behaviour of male offspring from PS and control rats was assessed in an elevated plus maze at PD 34 and in an open field arena at PD 35. Rats were then left in their home cages and tested for a 4-trial cocaine-induced CPP at PD 90: black arrows indicate saline (Sal) injections in the black compartment; white arrows indicate cocaine (Coc) or saline (Sal) injections in the white compartment, depending on the behavioural group (cocaine-treated or saline-treated, respectively). Animals treated with cocaine were classified according their CPP score in Low CPP and High CPP. Pubertal behaviour of these two groups of rats was then evaluated for PS and control offspring. CPP: conditioning place preference; EPM: elevated plus maze; GD: gestational day; Hab: CPP habituation; OF: open field; PD: postnatal day; Pre: CPP pretest; PS: prenatal stress group.

exposure to the box during the habituation day was performed to avoid different novelty-induced locomotor activation during pretest in PS rats respect to controls, thereby avoiding misinterpretations [38,48]. Time spent in each compartment during the second day (pretest) was used to determine each animal's compartment initial preference [45].

2.4.3.2. Conditioning. A biased conditioning procedure was used, where rats were conditioned with cocaine in the non-preferred (white) compartment and saline in the preferred (black) compartment [45,46,49]. Only one animal from C group showed no preference for the black side during pretest, but drug assignment was based on group preference and all animals received cocaine in the white less preferred side. The first conditioning day was always saline, i.e. all rats were injected with saline and immediately exposed to the preferred black compartment (door closed) for 30 min. The second conditioning day, rats were injected with 20 mg/kg (i.p.) cocaine (COC) and immediately exposed to the non-preferred white compartment (door closed) for 30 min. This entire procedure was repeated four times (four-trial CPP, Fig. 1). A saline control subgroup from PS and C groups received saline in both compartments (SAL). In order to evaluate acute and repeated cocaine-induced responsiveness, vertical and horizontal locomotor activity −expressed as number of rearing events and consecutive line breaks, respectively- were analysed throughout the whole conditioning session at the second and the last days of conditioning (i.e. the first and the fourth pairings in the white chamber) [46,50].

2.4.3.3. CPP test. 24 h following the last conditioning session, animals were tested in a drug-free state. Rats were allowed to explore the three compartments for 15 min with doors opened and time spent in each compartment was recorded for each animal. Time spent in each compartment was converted into a preference score [46,51]: [CPP score (sec) = time spent in the white compartment during test minus time spent in the white compartment during pretest].

2.4.4. Relationship between adult cocaine-induced CPP and pubertal behaviour

Cocaine-treated animals from C and PS groups were classified based on the magnitude of their CPP score in Low CPP (i.e. a CPP score below the mean minus SEM of the sample) or High CPP (i.e. a CPP score above the mean plus SEM of the sample). After that, results of pubertal behaviour were analysed for animals belonging to the Low CPP and High CPP groups separately (Fig. 1; [20]). Those animals with CPP scores between the mean ± SEM range were excluded from the analysis (n = 3 from PS group; Fig. 3a).Then, we had four groups: High CPP-PS rats (n = 7); High CPP-C rats (n = 7); Low CPP-PS rats (n = 5); and Low CPP-C rats (n = 5).

2.5. Statistical analysis

Schapiro-Wilk's and Levene's test were applied to verify data normal distribution and homogeneity of variances, respectively. The parameter "Time in open arms" in the elevated plus maze and "Grooming" in the open field were not normally distributed and were therefore transformed using square root and Log 10, respectively. Data were analysed by Student *t*-test, two-way ANOVA (Prenatal treatment x CPP group), or three-way repeated measures of ANOVA (with Pairing as within-factor) when appropriate. *Post hoc* comparisons were made by Scheffé test or by simple effects test when significant interactions between factors were found (InfoStat Software and SPSS Statistics v19). All results are expressed as mean + SEM. Significant differences were set at $p < 0.05$.

3. Results

3.1. Litter parameters on C and PS groups

In agreement with our previous studies [35,52,53], PS did not interfere with the length of gestation, number, ratio or weight of the pups at birth (Table 1). We found a significant increase in male body weight of PS offspring at PD 21 ($F_{1,15} = 5.897$; $p < 0.05$), according to previous reports which indicated that PS significantly increased the body weight of young male rats [39,54].

3.2. Pubertal behaviour in C and PS rats

Table 2 shows pubertal behaviour assessed in the EPM and the OF in C and PS offspring. In the EPM, Student *t*-test revealed no significant differences between C and PS groups for the time spent in open arms, the total number of entries to open or closed arms, the frequency of head dipping or the number of SAPs. In the OF, Student *t*-test revealed

Table 1
Litter parameters on control (C) and prenatally stressed (PS) groups. Results are expressed as mean ± SEM. Repeated measures of ANOVA; *$p < 0.05$ *vs.* C. PD: postnatal day.

Group	Length of gestation (days)	Litter Size		Weight of male pups (g)			
		Female Pups	Male Pups	PD 1	PD 7	PD 14	PD 21
C	22 ± 0	8 ± 1	6 ± 1	6,9 ± 0,3	15,3 ± 0,5	28,3 ± 1,3	41,3 ± 1,1
PS	22 ± 0	8 ± 1	6 ± 1	7,0 ± 0,1	15,7 ± 0,8	30,7 ± 1,5	47,1 ± 1,8*

Table 2
Pubertal behaviour assessed in control and prenatally stressed groups. Results are expressed as mean ± SEM. Student *t*-Test revealed no significant differences between C and PS groups; n = 29–32 per group.

	CONTROL	PRENATAL STRESS
Elevated plus maze		
Time in open arms (sec)	24.10 ± 3.19	29.06 ± 4.98
# Entries to open arms	3.24 ± 0.41	3.59 ± 0.49
# Head dipping	14.58 ± 2.05	13.87 ± 2.32
# Stretch attend posture	10.92 ± 1.30	8.93 ± 1.11
# Entries to closed arms	11.48 ± 0.66	12.94 ± 0.76
Open Field		
Total distance travelled (cm)	7425 ± 430	7485 ± 261
# Rearing	58.39 ± 6.47	60.73 ± 4.53
# Grooming	13.50 ± 1.72	9.73 ± 0.87

no significant differences between C and PS groups neither for total distance travelled nor for total number of rearing or grooming behaviour.

3.3. Cocaine-induced CPP during adulthood in C and PS rats

We investigated the influence of PS on cocaine effects in adult offspring using a 4-trial CPP. Fig. 2a shows the number of rearing events during the first and the last pairings with cocaine in the white chamber. Three-way repeated measures of ANOVA (Prenatal treatment x CPP group [SAL or COC] x Pairing, with Pairing as the repeated measure) revealed that there was a significant effect of CPP group ($F_{1,42} = 7.597$; $p < 0.01$) but not of Prenatal treatment factor. Within-subjects analysis revealed a significant effect for Pairing ($F_{1,42} = 36.093$; $p < 0.0001$) and for Pairing x CPP group ($F_{1,42} = 7.705$; $p < 0.01$). Simple effects test revealed that there were no significant differences for rearing behaviour between C and PS rats neither for the first nor for the last pairing in the white chamber. Cocaine-treated rats showed higher number of rearing than saline-treated rats only for C group at the first pairing ($p < 0.05$), although there was a trend for significance for PS group also at the first pairing ($p = 0.087$). Moreover, cocaine-treated rats showed a decreased rearing behaviour during the last pairing with the drug related to the first pairing ($p < 0.001$) regardless of prenatal treatment. Fig. 2b shows horizontal locomotor activity during the first and the last conditioning sessions in the white chamber in C and PS groups, analysed by three-way repeated measures of ANOVA ($F_{1;43} = 40.356$; $p < 0.0001$ for CPP group and $F_{1;43} = 2.969$; $p = 0.092$ for Prenatal treatment x CPP group). *Post hoc* comparisons by simple effects test revealed that cocaine-treated rats showed higher horizontal locomotor activity than saline-treated rats for C group ($p < 0.0001$) and for PS group ($p < 0.01$) during the first and the last pairings in the white chamber. Interestingly, C and PS cocaine-treated rats differed on their horizontal locomotor response to cocaine during the first pairing in the white chamber ($p = 0.011$), but not during the last one. Saline-treated animals showed no significant differences on horizontal locomotor activity, regardless of prenatal treatment. Fig. 2c shows CPP score in C and PS rats following cocaine conditioning. In our experimental conditions, two-way ANOVA ($F_{3,45} = 8.36$; $p = 0.0002$) followed by Scheffé *post hoc* test revealed a significant positive CPP only for PS rats, evidenced as a higher CPP score for cocaine-treated animals related to PS saline-treated controls ($p < 0.001$). Moreover, PS rats showed higher CPP score than C cocaine-treated rats ($p < 0.05$) and no significant differences were found between PS and C groups on saline-treated rats. Because of the lack of a significant positive CPP in C rats, we decided to further analyze our results, examining the effect of cocaine treatment on the preference for the drug-paired context (i.e. the white compartment). Supplementary Fig. 1 shows the time spent in the white compartment before and after conditioning, analysed by three-way repeated measures of ANOVA (prenatal treatment x CPP group x

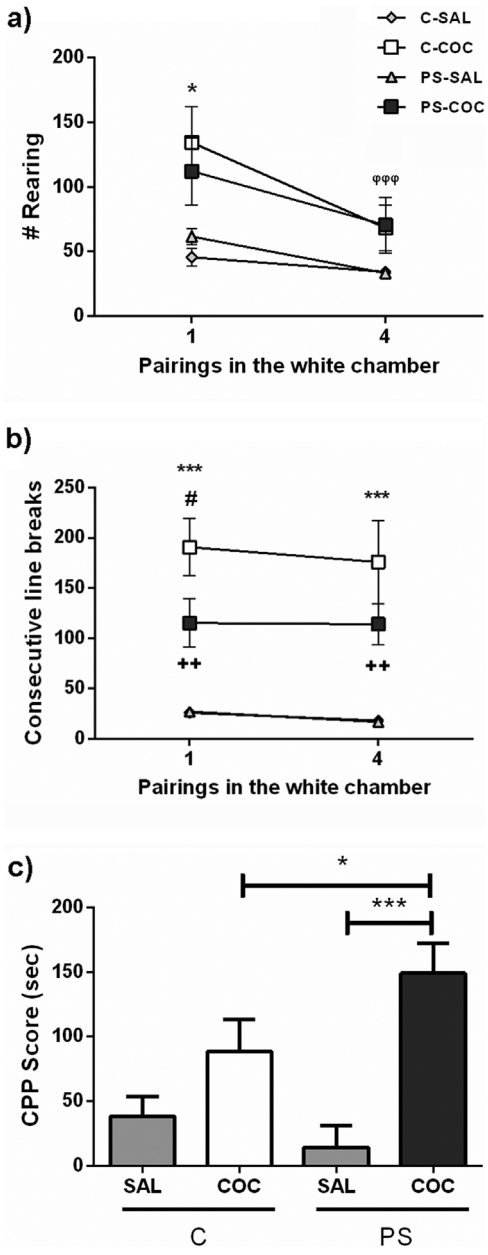

Fig. 2. Cocaine-induced conditioning place preference during adulthood in C and PS rats. Symbols indicate vertical exploration expressed as the number of rearing events **(a)** and horizontal locomotor activity expressed as the number of consecutive line breaks **(b)** exhibited during the first and the last conditioning sessions in the white chamber, for C and PS groups treated with cocaine or saline (n = 8–15 per group); $^{\varphi\varphi\varphi}p < 0.001$ pairing 4 *vs.* pairing 1 for C-COC and PS-COC; $^{*}p < 0.05$, $^{***}p < 0.001$ C-SAL *vs.* C-COC; $^{++}p < 0.01$ PS-SAL *vs.* PS-COC; $^{\#}p < 0.05$ C-COC *vs.* PS-COC. **c)** Bars indicate CPP score in cocaine-treated and saline controls from C and PS groups (n = 8–15 per group); $^{*}p < 0.05$, $^{***}p < 0.001$. Data are expressed as mean + SEM; C: control (non-prenatally stressed) rats; COC: cocaine-treated rats; CPP: conditioning place preference; PS: prenatally stressed rats; SAL: saline-treated rats.

[pretest *vs* test], with [pretest *vs* test] as the repeated measure: $F_{1,43} = 3.511$, $p = 0.06$ for CPP group; $F_{1,43} = 42.930$, $p < 0.001$ for [pretest vs test]; $F_{1,43} = 18.204$; $p < 0.001$ for CPP group x [pretest vs test]). Simple effects test revealed significant differences on the time spent in the white compartment between cocaine-treated and saline-treated rats for PS group ($p = 0.001$) but not for C group ($p = 0.399$). However, when analysing the time spent in the white compartment in test *vs* pretest, cocaine-treated rats from C and PS groups showed an increase on the preference for the drug-paired compartment following conditioning ($p < 0.001$). Moreover, during the test, PS cocaine-

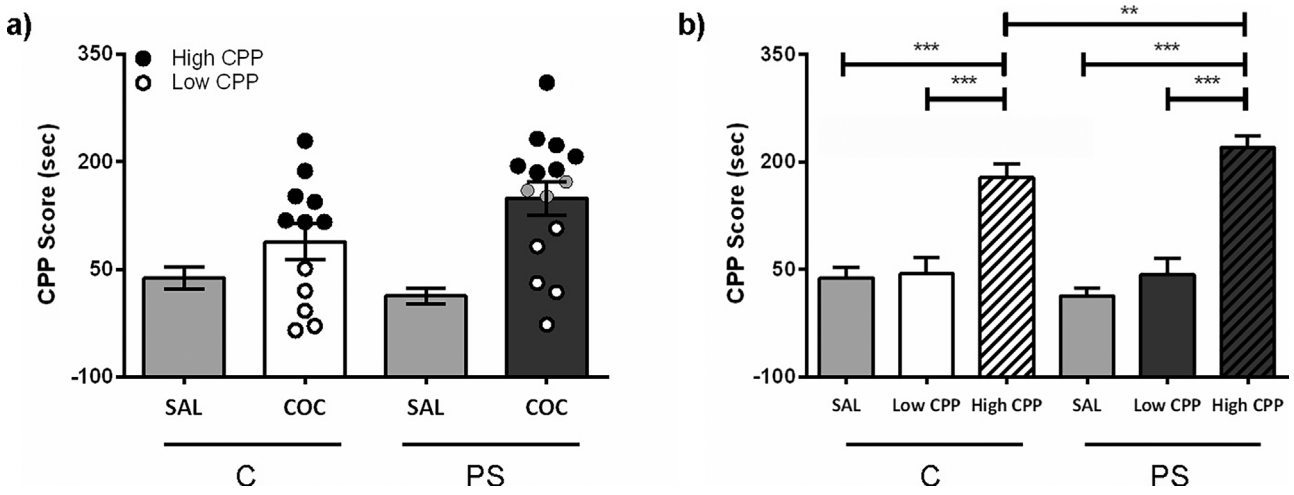

Fig. 3. Individual differences in cocaine-induced effects: Low and High CPP classification. **a)** Dot graph shows individual CPP score for cocaine-treated rats from C and PS groups according the mean ± SEM split criterion (see Section 2.4.4. for details). White and black dots represent those animals classified as Low CPP (n = 5, for C and PS groups) and High CPP (n = 7, for C and PS groups), respectively. Gray dots represent those animals which were excluded from the analysis (n = 3, from PS group). **b)** Bars indicate CPP score in C and PS saline- and cocaine-treated rats following Low CPP/High CPP classification. Data are expressed as mean ± SEM. $^{**}p < 0.01$; $^{***}p < 0.001$. C: control (non-prenatally stressed) rats; COC: cocaine-treated rats; CPP: conditioning place preference; PS: prenatally stressed rats; SAL: saline-treated rats.

treated rats showed a higher time spent in the white compartment than C cocaine-treated rats ($p = 0.020$), with no differences during pretest, confirming our main analysis in Fig. 2c.

3.4. Individual differences in cocaine-induced effects: Low and High CPP

In order to assess individual differences on cocaine-induced place conditioning, we classified cocaine-treated animals based on their CPP score in Low CPP or High CPP as shown in Fig. 3a (see Section 2.4.4 for details). When analysing CPP score in those groups (Fig. 3b), two-way ANOVA showed a significant effect for Prenatal treatment ($F_{1;38} = 4.65$; $p < 0.05$), for CPP group [SAL, Low CPP or High CPP] ($F_{1;38} = 70.22$; $p < 0.001$), and for the interaction between factors ($F_{2;38} = 5.41$; $p < 0.01$). Simple effects test revealed that only High CPP rats exhibited a positive CPP compared with saline control, regardless of prenatal treatment ($p < 0.001$). Moreover, High CPP-PS rats showed a higher CPP score than High CPP-C rats ($p < 0.01$). No significant differences were observed between Low CPP and saline control groups from C or PS groups.

3.5. Differential pubertal behaviour in Low CPP and High CPP offspring

Following Low CPP and High CPP classification, we re-analysed the results of pubertal behaviour in cocaine-treated rats from C and PS groups (see Fig. 1 and Section 2.4.4. for details). The saline groups were not included in the following analysis.

3.5.1. Anxiety-like behaviour

Fig. 4 shows pubertal behaviour assessed in the EPM for C and PS rats classified as Low CPP or High CPP. For PS group, our results showed that High CPP spent more time ($F_{1;20} = 6.07$; $p < 0.05$; Fig. 4a) and showed a higher number of entries to the open arms of the EPM than Low CPP rats ($F_{1;20} = 4.39$; $p < 0.05$; Fig. 4b). Between C and PS groups we found that Low CPP-C rats spent more time in open arms ($F_{1;20} = 5.95$; $p < 0.05$; Fig. 4a) and showed a higher number of entries to open arms ($F_{1;20} = 6.71$; $p < 0.05$; Fig. 4b) than Low CPP-PS rats. For C group, there were no significant differences on time spent in open arms or number of entries to open arms between Low CPP and High CPP. Fig. 4c and d shows additional pubertal behavioural parameters assessed in the EPM, for C and PS rats classified as Low CPP or High CPP. High CPP-PS rats showed a higher number of head dipping events than Low CPP-PS rats ($F_{1;20} = 6.00$; $p < 0.05$; Fig. 4c). For C group, there were no significant differences on head dipping behaviour. Our results also showed that Low CPP-C rats protruded the head towards the floor more times than Low CPP-PS rats ($F_{1;20} = 4.50$; $p < 0.05$; Fig. 4c). There were no significant differences intra C or PS groups or between C and PS groups for stretching behaviour (Fig. 4d).

3.5.2. Locomotor response to novelty

Fig. 5 shows pubertal novelty response in C and PS cocaine-treated rats classified as Low CPP and High CPP. There were no significant differences between groups neither for the total distance travelled (Fig. 5a), nor for the number of rearing (Fig. 5b) or grooming (Fig. 5c) behaviours during the 20 min session in the OF. A trend towards significance was evidenced for Prenatal treatment on grooming behaviour ($p = 0.0751$).

4. Discussion

Early-life stress may predispose individuals to develop neuropsychiatric diseases later in life, including mood disorders and SUD [55]. A major goal of this study was to assess the impact of PS on pubertal behaviour and adult vulnerability to cocaine-induced reward.

For the present study, we assessed cocaine reward using the CPP task because it has several advantages: 1) CPP allows the quantification of the degree of drug-induced reward with few exposures [22,46]; 2) locomotor sensitization during conditioning can be quantified [46,56]; 3) the preference is assessed in a drug-free state, thus avoiding interferences due to drug-induced locomotor activity [57–60]; and 4) it allows identification of individual differences in the strength of drug-induced CPP [20]. Currently, there are relatively few studies that have examined sex differences in SUD vulnerability induced by PS [17,18]. In this study, we assessed cocaine reward in male offspring due to our previous findings showing neurobiological changes in dopamine circuits in male rats [61,62]. Nevertheless, we are aware of the influence of sex on the addiction process (reviewed by [63]) and the evaluation of sex differences on PS-induced SUD vulnerability should be included in future research.

4.1. Prenatal stress-related behaviours

Several studies have assessed different behavioural traits in PS individuals. Most authors, including ourselves, showed that adult PS male offspring are more anxious [39,64–67] and more active when exposed

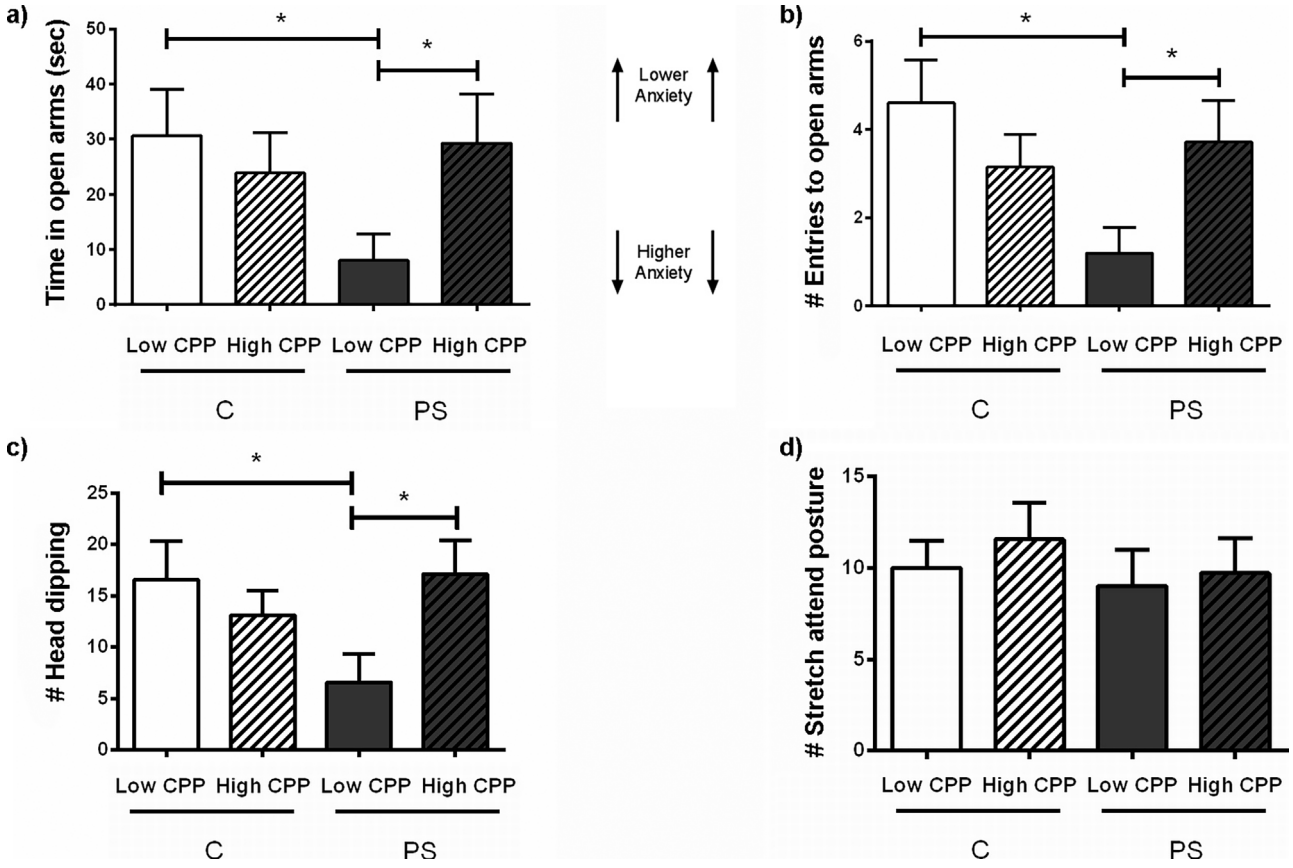

Fig. 4. Analysis of pubertal anxiety-like behaviour in rats classified as Low CPP and High CPP. Cocaine-treated animals during CPP were classified in Low CPP or High CPP depending on their CPP score and then we analysed their pubertal anxiety-like behaviour. During the 5 min assay in the elevated plus maze we quantified the time spent in open arms **(a)**, total number of entries to open arms **(b),** the number of head dipping **(c)** and stretch attend posture events **(d)**. Higher levels of anxiety are represented by less time spent or less number of entries to open arms. Data are expressed as mean ± SEM (n = 5–7 per group); $^*p < 0.05$. C: control (non-prenatally stressed) rats; CPP: conditioning place preference; PS: prenatally stressed rats.

to a novel environment [48] than control rats. However, other authors showed that PS decreased or did not affect anxiety-like behaviour and did not influence novelty response [54,68]. Regarding stretching and head dipping measures in the EPM, Estanislau and Morato [39] have reported that PS had no effects on stretching behaviour, while it induced a decrease on the frequency of head dipping in the open arms, only for adult rats. In spite of potentially representing behavioural traits anticipatory of later SUD vulnerability, few studies have assessed these stress-related behaviours during puberty, even though it has been suggested that PS effects on emotional responsiveness emerge at late adolescence [39,69]. In this study, we found that locomotor response to novelty was similar in pubertal C and PS rats and, in agreement with Estanislau and Morato [39], we also found no differences in anxiety-like behaviour. In contrast, Vey et al. [38] reported a decreased time spent in open arms in the EPM in adolescent (PD39) offspring of dams exposed to a variable and longer unpredictable stress protocol. These differences could be explained considering that different prenatal protocols inflicted to the pregnant dam could affect differently the offspring behaviour later in life [70].

4.2. Prenatal stress and SUD

Preclinical data of SUD employing different protocols such as self-administration [17,19,48,71] or CPP [38,40,51,72] have shown that PS

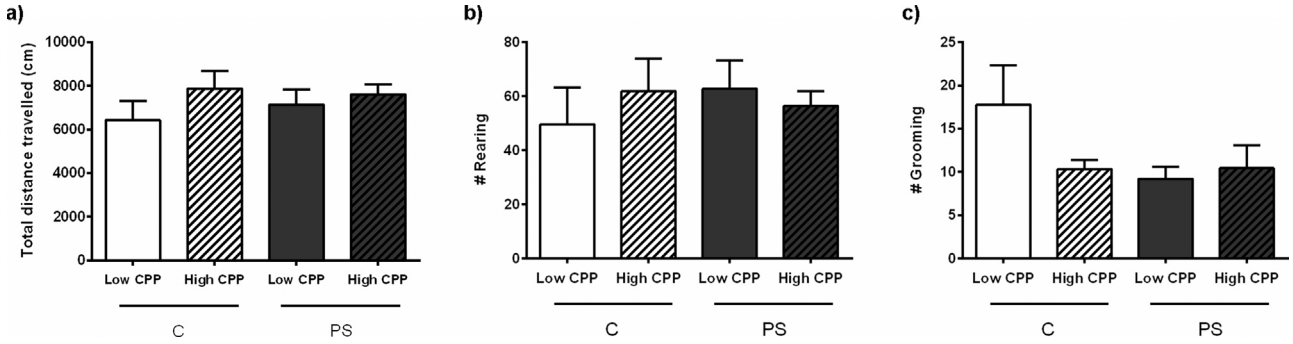

Fig. 5. Analysis of pubertal novelty response in rats classified as Low CPP and High CPP. Cocaine-treated animals during CPP were classified in Low CPP or High CPP depending on their CPP score and then we analysed their pubertal locomotor response to novelty. There were no significant differences between Low CPP and High CPP or between C and PS groups neither for total distance travelled **(a)** nor for number of rearing **(b)** or grooming **(c)** behaviours. Bars indicate mean ± SEM (n = 5-7 per group). C: control (non-prenatally stressed) rats; CPP: conditioning place preference; PS: prenatally stressed rats.

could increase the vulnerability to develop addictive-like behaviours in the offspring. In this study, we found that PS significantly increased cocaine-induced CPP in adult rat offspring in accordance with previous results obtained in B6 mice [18]. Moreover, we found that PS rats exhibited lower horizontal locomotor activity than C rats during the first pairing with cocaine in the CPP box, in agreement with previous studies showing that a lower locomotor response to an acute psychostimulant administration is related to a higher drug-induced conditioning place preference [46,56,73]. A simple explanation to this negative association could be that cocaine-induced locomotor activity interferes with the conditioning process (i.e. the association between drug effects and environmental cues). However, we found no significant differences on vertical exploration (i.e. number of rearing events in the white chamber) between C and PS cocaine-treated rats and, more importantly, PS and C rats differed in cocaine-induced horizontal locomotor activity only during the first pairing and no major locomotor sensitization was evidenced following conditioning sessions. Those differences in locomotor activity during conditioning could be related to PS-induced neuroadaptations in the dopamine system [16,61,74] leading, for example, to different dopamine clearance in nucleus Accumbens due to the inhibition of the dopamine transporter by cocaine, as suggested by Sabeti et al. [75]. Further research is needed to unravel molecular mechanisms underlying those changes.

4.3. Behavioural traits and SUD: the influence of PS

Adult anxiety disorders and SUD have been strongly associated in both humans [76–80] and animal models [20–22]. However, the relationship between anxiety-like behaviour and the rewarding or reinforcing effects of cocaine assessed by CPP or self-administration protocols is inconsistent across rodent studies, with some of them showing a positive relationship [20–25] and others showing a negative or a lack of association [26–28]. The relationship between novelty response and CPP also shows some inconsistencies in the literature, since several authors claimed that novelty response is related to the magnitude of cocaine-induced CPP ([81]; reviewed by [82–84]), whereas other authors showed that individual differences in locomotor response to a novel environment are not related to the strength of cocaine-induced CPP [20,85–88]. In addition to the fact that those studies used different protocols to assess cocaine reward or reinforcement, it is feasible that different early life experiences had affected the pups and inadvertently interfere with the results, especially considering that individual differences in response to cocaine are likely due to ontogenetic differences in brain regions related with the reward system [29].

Moreover, it is interesting to notice that in previous studies both anxiety trait/novelty response and cocaine effects were assessed during adulthood. We design the present study to assess anxiety-like behaviour and novelty response at puberty, considering that it could be a useful approach not only to examine their relationship with an increased adult SUD vulnerability, but also for providing an opportunity to intervene before the establishment of the pathology. In this study, we found that PS increased cocaine-induced CPP with no effects on pubertal anxiety-like behaviour or locomotor response to novelty. However, it is interesting to notice that PS may or may not affect the offspring by making it vulnerable or resilient to future behavioural alterations in response to trigger factors such as drugs of abuse (reviewed by [89]). Therefore, we divided the adult offspring in resilient (Low CPP) or vulnerable (High CPP) and analysed a retrospective link between the magnitude of cocaine effects and pubertal behaviour in C and PS rats, taking the advantage that animal behaviour, unlike human studies, can be evaluated retrospectively without the possibility of false self-reports [90].

For C group, we found no relationship between pubertal anxiety and adult vulnerability to cocaine reward. However, for PS group, we found that High CPP rats exhibited lower anxiety-like behaviour during puberty, indicating that the relationship between pubertal anxiety and adult vulnerability to cocaine reward assessed by CPP depends on the exposure of the offspring to PS. To our knowledge, this is the first study showing that pubertal anxiety-like behaviour is related to adult cocaine-induced CPP, an interesting approach considering that pubertal detection of behavioural traits lies in the possibility of early intervention that will allow the development of prevention strategies to avoid the devastating consequences of drug addiction later in life. Recently, it has been proposed that individual differences on emotional behaviour could be related to a successful or unsuccessful adaptation to early life adversities [91]. Moreover, certain behavioural traits (i.e. increased anxiety) could be maladaptive and increase SUD risk but also, they could be adaptive in the context of an animal exposed to prenatal stress [92]. In this work, Low CPP-PS rats had shown higher levels of anxiety-like behaviour than Low CPP-C rats, supporting that behavioural traits cannot be considered “good or bad” *per se* but the risk to develop a SUD depends on the adaptive or maladaptive capacity of the organism when confronted to the environment [92]. According to Homberg et al. [92], the organisms thrive when the environment match their traits and genotype. However, under non-match conditions, the individuals need to compensate the failure and the rewarding properties of drugs of abuse might help to alleviate this mismatch.

In summary, our results underline the importance of assessing individual differences to cocaine reward when looking for behavioural traits related to vulnerability in PS rats. In our hands and with our paradigm, we clearly showed that anxiety-like behaviour assessed in an EPM but not novelty response assessed in an open field arena, is a behavioural trait related to the vulnerability to cocaine-induced place preference in PS rats. These findings also highlight the importance of knowing if an individual was exposed to PS when assessing behavioural traits in the search for screening methods for identifying at-risk individuals. Considering that PS induces long- term epigenetic changes [93,94], those alterations should be explored in future research to assess molecular mechanisms underlying these behavioural findings and, more importantly, for the development of early biomarkers of PS exposure.

5. Conclusion

As previously reviewed by our group and others [14–16,61], there is nowadays no doubt that the exposure to stress during pregnancy can alter the proper development of neural circuits in the offspring, increasing the propensity to develop neuropsychiatric diseases, including SUD. This study provides the first evidence suggesting that individual differences in cocaine-induced CPP are related to pubertal anxiety-like behaviour in prenatally stressed rats. In conclusion, identifying risk factors that appear early in development as well as considering prenatal exposure to stress are crucial for detecting vulnerable individuals thus allowing timely prevention strategies that will avoid SUD development later in life.

Conflicts of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bbr.2017.11.035.

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