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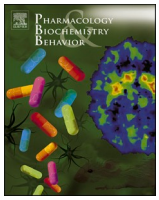

Resilience to the short- and long-term behavioral effects of intermittent repeated social defeat in adolescent male mice

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ABSTRACT

Background: Exposure to intermittent repeated social defeat (IRSD) increases the sensitivity of mice to the rewarding effects of cocaine in the conditioned place preference (CPP) paradigm. Some animals are resilient to this effect of IRSD, though research exploring this inconsistency in adolescent mice is scarce. Thus, our aim was to characterize the behavioral profile of mice exposed to IRSD during early adolescence and to explore a potential association with resilience to the short- and long-term effects of IRSD.

Methods: Thirty-six male C57BL/6 mice were exposed to IRSD during early adolescence (PND 27, 30, 33 and 36), while another 10 male mice did not undergo stress (controls). Defeated mice and controls then carried out the following battery of behavioral tests; the Elevated Plus Maze, Hole-Board and Social Interaction Test on PND 37, and the Tail Suspension and Splash tests on PND 38. Three weeks later, all the mice were submitted to the CPP paradigm with a low dose of cocaine (1.5 mg/kg).

Results: IRSD during early adolescence induced depressive-like behavior in the Social Interaction and Splash tests and increased the rewarding effects of cocaine. Mice with low levels of submissive behavior during episodes of defeat were resilient to the short- and long-term effects of IRSD. In addition, resilience to the short-term effects of IRSD on social interaction and grooming behavior predicted resilience to the long-term effects of IRSD on cocaine reward.

Conclusion: Our findings help to characterize the nature of resilience to the effects of social stress during adolescence.

1. Introduction

Adolescence is a critical developmental stage characterized by physical, emotional, cognitive and behavioral changes during which the process of brain maturation is completed (Spear, 2000). For this reason, the adolescent brain is particularly sensitive to stressors (Romeo, 2017). Studies in animal models have demonstrated that exposure to stress during adolescence alters brain development, increasing vulnerability to the development of anxiety, depression and drug use disorders in adulthood (al'Absi, 2020; Burke et al., 2017). While chronic stress is generally associated with the appearance of psychopathologies, recent studies have shown that, under certain circumstances, exposure to stress in early life or adolescence can promote resilience to future stressful events (Calpe-López et al., 2022a; Cotella et al., 2022; Mancini et al., 2021; Ordoñes Sanchez et al., 2021).

In previous works, we have studied the long-term consequences of social stress on the vulnerability of mice to drugs of abuse by exposing animals to a protocol of intermittent repeated social defeat (IRSD) by an aggressive adult conspecific animal. This paradigm has been demonstrated to be useful for modelling social stress, which is the most frequent type of stress faced by human beings (Calpe-López et al., 2022a, 2022b, 2022c; García-Pardo et al., 2015, 2022). Mice are usually exposed to defeat during late adolescence or adulthood; however, the use of early adolescent mice may be of greater relevance, as it models the human context in which bullying takes place. We have previously demonstrated that exposure to IRSD in early or late adolescence

Abbreviations: CPP, *conditioned place preference*; *EPM*, *elevated plus maze*; *FG*, *frequency of grooming*; *ISI*, *index of social interaction*; *NS*, *novelty-seeking*; *PND*, *post-natal day*; *IRSD*, *intermittent repeated social defeat*; *TG*, *time in grooming*; *TI*, *time of immobility*; *TST*, *tail suspension test*; *%TOA*, *percentage of time in the open arms.*.

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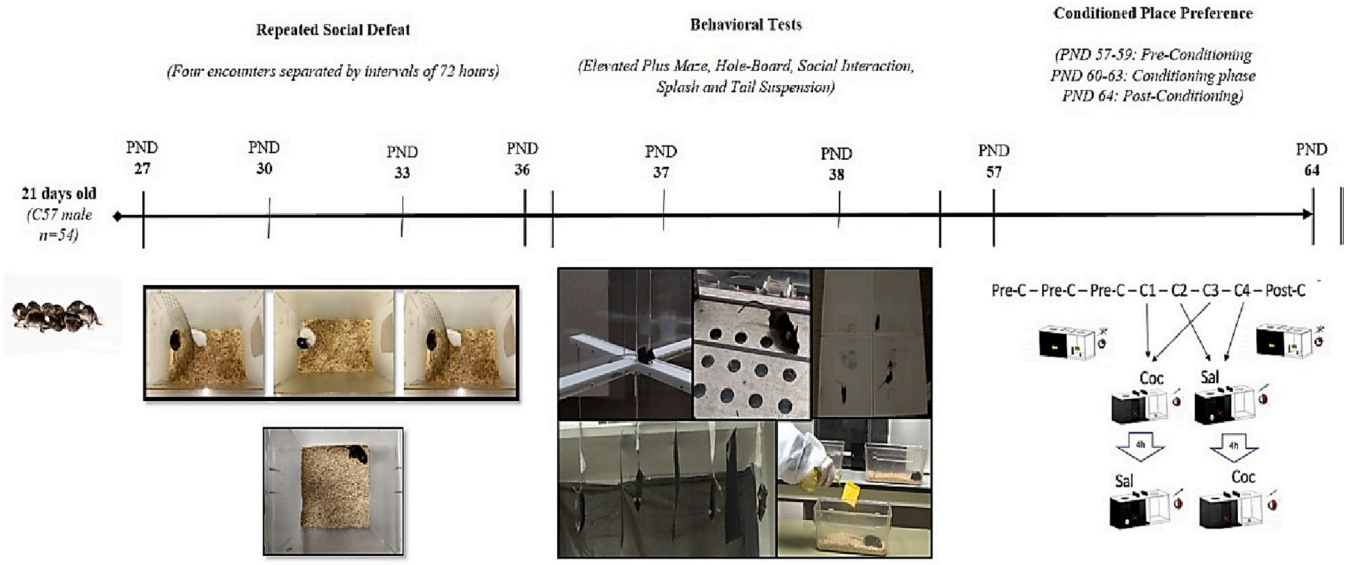

Fig. 1. Experimental Design. Two groups of mice were used. One group was exposed to intermittent repeated social defeat (IRSD, $n = 36$). On postnatal day (PND) 27, 30, 33 and 36, experimental mice were introduced into the cage of an aggressive opponent. The physical contact between them was allowed for 5 min, during which the experimental mouse experienced social defeat (SD). On the same PND, the mice in the other group (CONTROL, $n = 10$) explored an empty cage. On PND 37, all mice performed the elevated plus maze (EPM), the hole board (HB) and social interaction (SI) tests. On PND 38, all mice performed the tail suspension test (TST) and the splash test. After an interval of 3 weeks, all mice underwent the conditioned place preference (CPP) paradigm. On PND 57, 58 and 59, they underwent the pre-conditioning (Pre-C) phase. On PND 60, 61, 62, 63 they performed four conditioning sessions (C1-C4), receiving 1.5 mg/kg of cocaine (Coc) or saline (Sal) before being placed in the drug- or saline-paired compartment, respectively. On PND 64, mice underwent the post-conditioning (Post-C) phase.

enhances the vulnerability of mice to the rewarding effects of cocaine (Calpe-López et al., 2020; Montagud-Romero et al., 2017; Rodríguez-Arias et al., 2017) and MDMA (García-Pardo et al., 2015) in the conditioned place preference (CPP) paradigm.

As explained previously, an increasing number of studies are focusing on resilience to the effects of stress rather than vulnerability (Calpe-López et al., 2022a). As in humans, not all mice exposed to social stress develop depression-, anxiety- or addictive-like disorders (Krishnan et al., 2007; Russo et al., 2012; Schmidt et al., 2010). In a previous study we observed an increase in the rewarding effects of cocaine in adulthood in a subset of mice exposed to IRSD during late adolescence (susceptible mice), while another subset showed resilience to the negative effects of stress on cocaine reward (Calpe-López et al., 2020). In the same study we demonstrated that vulnerability and resilience to the effects of IRSD on late adolescence were associated with different behavioral profiles. Resilient mice were characterized by less submission during defeat episodes, less interest in the open arms in the elevated plus maze (EPM), less novelty-seeking in the hole-board test, less reactivity in the tail suspension test (TST), and an absence of IRSD-induced deficits such as social avoidance in the social interaction test and anhedonia in the splash test (Calpe-López et al., 2020).

Resilience to the negative consequences of social defeat stress in early adolescence has been less studied. It has been suggested that the responses of adolescent mice exposed to different paradigms of social defeat are more complex and singular in comparison to those of mice defeated in adulthood. Adult mice exposed to chronic social defeat stress (CSDS) can be characterized according to their levels of social interaction and sucrose preference as susceptible or resilient to CSDS (Krishnan et al., 2007; Russo et al., 2012). Conversely, it has been observed that only a small proportion of mice defeated in adolescence were either totally susceptible or totally resilient in both social interaction and sucrose preference tests, while most were susceptible in one test and resilient in the other (Alves-Dos-Santos et al., 2020). Similar age differences seem to exist in resilience to the effects of IRSD. Adult mice exposed to IRSD showed a consistent resilient phenotype to depressive-like behaviors (in the social interaction and splash tests) and to an increase in cocaine reward (Ballestín et al., 2021; Calpe-López et al., 2020) and alcohol consumption (Reguilón et al., 2021). However, mice exposed to IRSD during adolescence and which displayed resilience to depressive-like behaviors in the social interaction test exhibited more anxiety in the EPM, an increased preference for cocaine-paired compartment and greater ethanol consumption (Reguilón et al., 2022).

Thus, the aim of this work was to characterize the behavioral profile of mice exposed to IRSD during early adolescence and to evaluate the potential association of this profile with resilience to the short- and long-term effects of IRSD. We have followed exactly the same procedure as that used in a previous study with late adolescent mice (Calpe-López et al., 2020), the only difference being the post-natal days (PND) on which defeat was experienced. In this way, we could compare the phenotype of resilient mice exposed to IRSD in early (PND 27–36) versus late (PND 47–56) adolescence.

2. Material and methods

2.1. Subjects

A total number of 46 male mice of the C57BL/6 strain and 15 male mice of the OF1 strain (Charles River, France) were delivered to our laboratory at 21 days of age and 42 days of age, respectively. Experimental mice (C57BL/6) were housed in groups ($n = 4–5$) while mice used as aggressive opponents (OF1) were housed individually to induce heightened aggression (Rodríguez-Arias et al., 1998). All mice were housed under standard laboratory conditions (see details in Calpe-López et al., 2020). All procedures were conducted according the Directive 2010/63/EU and were approved by the Ethics Committee of Experimental Research of the University of Valencia (A1507028485045).

2.2. Drugs

Animals were injected intraperitoneally with 1.5 mg/kg of cocaine (Alcaliber Laboratory, Madrid, Spain) or physiological saline (NaCl 0.9 %) in a volume of 0.01 ml/g of weight. Physiological saline was also used to dissolve the cocaine. The dose of cocaine was selected on the basis of a study evaluating resilience to the effects of IRSD in early

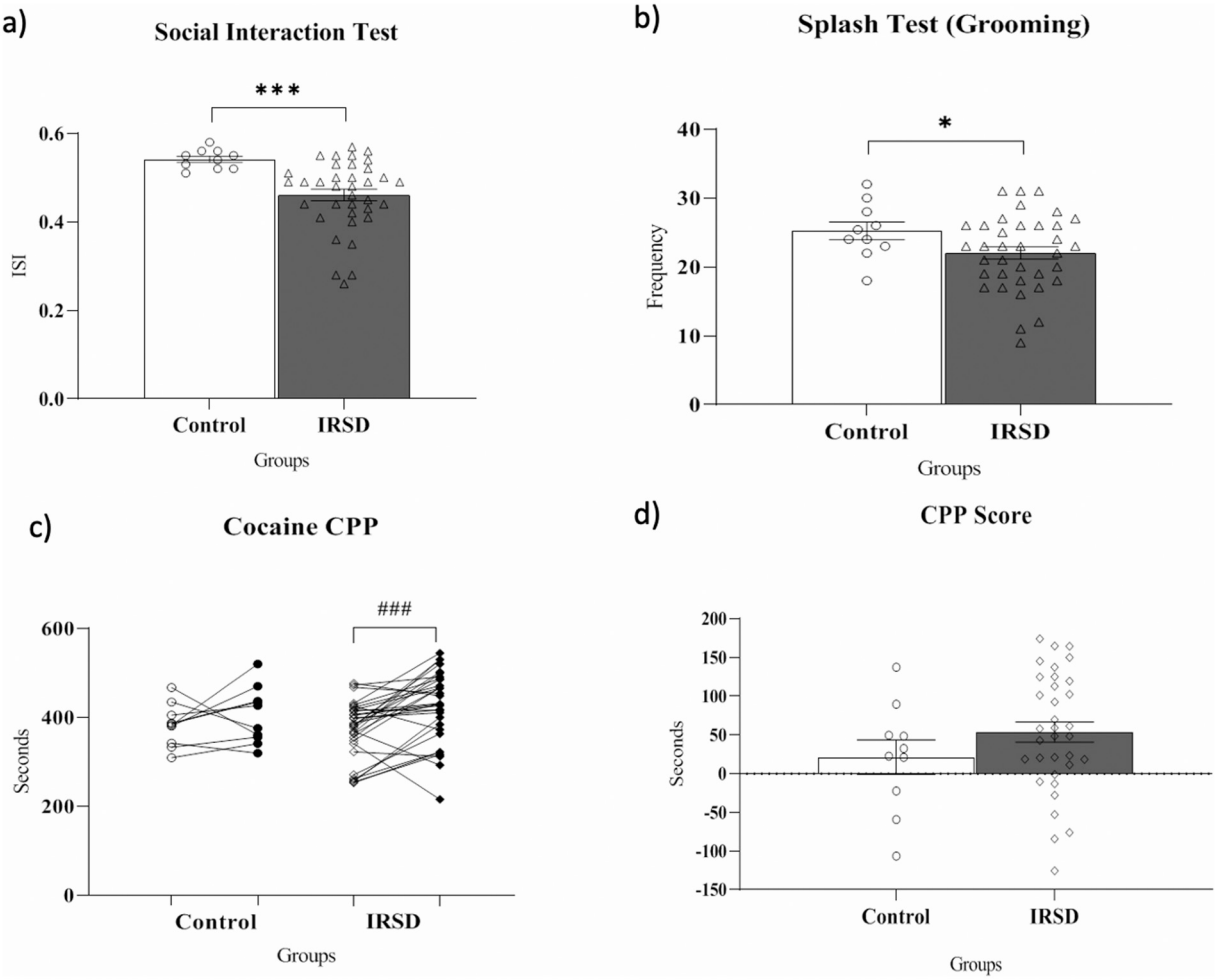

Fig. 2. Behavioral effects of IRSD. One group of early adolescent male mice was not exposed to stress (CONTROL, $n = 10$) and the other group was exposed to IRSD on PND 27, 30, 33 and 36 ($n = 36$). a) Short-term effects of IRSD on the social interaction test. Bars represent the mean (±SEM) ISI. ***p < 0.001, significant difference with respect to the CONTROL group. b) Short-term effects of IRSD on the frequency of grooming behavior in the splash test. *$p < 0.05$, significant difference with respect to the CONTROL group. c) Long-term effects of IRSD on cocaine-induced CPP. All mice (CONTROL and IRSD groups) were conditioned with 1.5 mg/kg of cocaine. Lines represent the time (in seconds) spent in the drug-paired compartment in the pre-conditioning test (Pre—C; empty symbols) and in the post-conditioning test (Post-C; filled symbols). ### p < 0.001, significant difference in the time spent in the drug-paired compartment in Post-C vs. Pre-C test. d) CPP score in control and IRSD groups. Bars represent the mean (±SEM) time (in seconds) spent in the drug-paired compartment in Post-C minus that spent in the same compartment in Pre—C.

adolescent mice on cocaine CPP (Reguilón et al., 2022). We used this dose of cocaine because it is ineffective in naïve animals (as we confirmed in pilot studies), thus allowing us to detect a potentiation of drug reward in mice exposed to IRSD, which we expected would acquire cocaine CPP.

2.3. Experimental design

Following the adaptation period, the experimental mice (C57BL/6) were assigned to two groups: a non-stressed control group ($n = 10$) and a group subsequently exposed to four episodes of IRSD ($n = 36$) on PND 27, 30, 33 and 36. On PND 37–38, all the mice underwent different behavioral tests to evaluate the short-term effects of IRSD (see Fig. 1). The effects of IRSD on anxiety-like behavior were evaluated in the elevated plus maze (EPM), a test based on the aversion of mice to open elevated areas and their spontaneous exploration of novel environments. A reduction in the number or percentage of entries into the open arms and in the time spent (or percentage of time) in the open arms are commonly considered to be indicators of anxiety (Campos et al., 2013). The effects of IRSD on the novelty-seeking behavior of our mice were evaluated in the hole board (HB) test by measuring the number of dips they performed (exploration of the holes). The effects of IRSD on social interaction were evaluated in the social interaction test by comparing the time that the experimental mice spent in an interaction zone in the absence or presence of another mouse (Henriques-Alves and Queiroz, 2016; Krishnan et al., 2007). The effects of IRSD on depression-like behavior were evaluated by the tail suspension test (TST), which measures the behavioral variable of immobility, considered to represent despair (Pollak et al., 2010); and by the splash test, in which a decrease of grooming is interpreted as anhedonia (Brachman et al., 2016; de Souza et al., 2019; Smolinsky et al., 2009). The animals were then housed in the vivarium for 3 weeks, after which they underwent an unbiased CPP procedure (PND 57–64) to evaluate the conditioned rewarding effects of cocaine (see Fig. 1). All experiments took place during the dark period (8.30–16.30) and in a different environment to that of the confrontation sessions. In order to facilitate adaptation, mice were transported to the dimly illuminated experimental room 1 h prior to testing. All the experimental protocols were performed as is described in Calpe-López et al. (2020). In addition, detailed information of the experimental protocols can be found in the Supplementary material.

Table 1
Behavioral categories evaluated during the first and fourth episode of social defeat in experimental and opponent mice. Defeated mice were classified as Low or High Submission according to the time spent in Defense/Submission behavior in the first defeat. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$, significant difference with respect to IRSD Low Submission group.

			1st SD		4th SD	
			IRSD low submission	IRSD high submission	IRSD low submission	IRSD high submission
Experimental (intruder) mice	Defense/Submission	Frecuency	7,55 (± 1)	14,68 (± 0,95) +++	8,61 (± 0,95)	12,44 (± 1,21) ++
		Latency	28,47 (± 6,8)	9,79 (± 2,57) ++	19,02 (± 4,41)	24,93 (± 8,66)
		Time	29,61 (± 3,73)	79,72 (± 7,55) +++	44,4 (± 6,85)	66,9 (± 7,3) ++
	Avoidance/Flee	Frecuency	12,56 (± 1,29)	15,5 (± 1,84)	12,28 (± 0,99)	11,94 (± 1,02)
		Latency	9,32 (± 4,53)	7,16 (± 4,09)	5,93 (± 3,95)	1,85 (± 0,68)
		Time	90,61 (± 11,67)	69,92 (± 10,91)	73,25 (± 8,92)	62,52 (± 6,31)
Opponent (resident) mice	Threat	Frecuency	8,56 (± 0,87)	11,22 (± 0,79) +	5,95 (± 0,89)	9,21 (± 1,31) +
		Latency	5,58 (± 2,77)	8,34 (± 3)	30,03 (± 15,27)	27,54 (± 10,27)
		Time	50,68 (± 14,08)	83,47 (± 15,13)	29,6 (± 10,07)	48,23 (± 6,54)
	Attack	Frecuency	7,61 (± 1)	7,06 (± 1,15)	7,94 (± 0,78)	6,78 (± 1,08)
		Latency	27,69 (± 8,1)	55,83 (± 17,87)	21,22 (± 13,14)	44,25 (± 18,9)
		Time	34 (± 5,03)	29,43 (± 4,34)	41,12 (± 5,32)	35,6 (± 5,69)

2.4. Statistical analysis

The effects of IRSD on the different behavioral measures (with the exception of CPP) were evaluated by means of unpaired Student *t*-tests (Control vs. IRSD). In the case of CPP, a mixed two-way ANOVA with a within-subjects variable – Days, with two levels (Pre-C and Post-C) - and a between-subjects variable – Stress, with two levels (Control and IRSD) - was used. Post hoc comparisons were performed with Simple Effects and Bonferroni tests.

With the data obtained in the defeat episodes and in the behavioral tests (EPM, hole board, social interaction, tail suspension and splash tests), the group of defeated mice was separated into two subgroups (High or Low Score group) according to the median of the whole group (see detailed information in Calpe-López et al. (2020) and Supplementary Material). Unpaired Student's *t*-tests were performed between mice showing High and Low submissive behavior (time in defense/submission in the first episode of defeat), for the behavior shown during the first and fourth episodes of defeat, and for the measurements of percentage of time in the open arms of the EPM, number of dips in the hole board, ISI, time immobile in the TST, and grooming (frequency and time) in the splash test. To determine the influence of the different behavioral profiles on resilience to the short-term behavioral effects of IRSD, a one-way ANOVA with a between-subjects variable—Group, with three levels (Control, IRSD High Score and IRSD Low Score)—was performed for the percentage of time spent in the open arms of the EPM, number of dips in the hole board, ISI, time immobile in the TST, and grooming (frequency and time) in the splash test. The post hoc comparisons were performed with Bonferroni tests.

To determine the possible behavioral markers of resilience to the effects of social defeat on cocaine CPP, a mixed two-way ANOVA with a within-subjects variable—Days, with two levels (Pre-C and Post-C)—and a between-subjects variable—Group, with three levels (Control, IRSD High Score and IRSD Low Score for all the measures described above)—was performed with data observed in the CPP paradigm. Post hoc comparisons were performed with Bonferroni tests. In order to determine whether there was a relationship between the performance of mice in the short-term behavioral tests and in the CPP, Pearson correlation tests were used (see Supplementary material). For this purpose, the conditioning score (time spent in Post-C minus time spent in Pre—C) was calculated. In addition, linear regression analyses comparing the different behavioral measurements were performed (see Supplementary material). All statistical analyses were performed with the SPSS program.

3. Results

3.1. Behavioral effects of IRSD

Regarding the short-term behavioral effects of IRSD, the Student *t*-test comparing Control and IRSD-exposed mice revealed that the experience of IRSD during early adolescence only induced a significant deficit in the ISI [$t(44) = 5.342$; $p < 0.001$; $d = 1.17$] (see Fig. 2a) and a reduction in frequency of grooming [$t(44) = 1.711$; $p < 0.05$; $d = 0.61$] (see Fig. 2b). No significant differences were observed in the measurements obtained in the EPM, hole-board and tail suspension procedures.

Regarding the long-term effects of IRSD on cocaine reward, ANOVA of the CPP data showed that the variable Days was significant [$F(1,40) = 8.549$; $p < 0.01$; $\eta p^2 = 0.18$], while the variables Stress and the interaction Days x Stress were not. It is likely that this interaction was not significant due to the fact that both groups were conditioned with cocaine, which would have induced a similar effect in both groups (i.e., an increase in the time spent in the drug-paired compartment in Post-C vs. Pre—C). However, as can be seen in Fig. 2c and d, the extent of this increase clearly differed, and was more pronounced in mice exposed to IRSD. For this reason, we decided to perform a simple effects analysis of the variable Days in the Control and IRSD groups. The simple effects revealed that the increase in the time spent in the drug-paired compartment between Pre- and Post-C days was significant only in the group of mice exposed to IRSD [$F(1,40) = 18.375$; $p < 0.001$; $\eta p^2 = 0.32$] (see Fig. 2c).

In addition, based on the data obtained in the present study (see Fig. 2d) and on the criterion used by Ma et al. (2012), we decided to apply a criterion of ±50 s of difference between Pre-C and Post-C values as an indication of the development of preference or aversion. In the control group ($n = 10$) we observed that 2 mice (20 %) developed preference and 2 mice (20 %) developed aversion. In the case of the IRSD group ($n = 32$), 17 mice (53 %) developed preference, while 4 mice (12,5 %) showed aversion.

3.2. Behavioral profile of mice during social defeat and resilience to the effects of IRSD

According to their scores in the time spent engaged in Defense/Submission behavior during the first episode of defeat, defeated mice were separated into two subgroups as mice with Low or High Submissive behavior (below or above the median of the defeated group, which was 55 s; see Table 1). Student *t*-test showed that these groups differed significantly regarding the time spent in defense/submission [$t(25) = -6.374$; $p < 0.001$; $d = -2.13$], and the frequency [$t(34) = -5.676$; $p < 0.01$; $d = -1.89$] and latency [$t(34) = 2.564$; $p < 0.01$; $d = 0.86$] of this behavior in the first episode of defeat. In addition, Low Submissive mice

Table 2
Measurements obtained in the behavioral tests performed short-term after IRSD in defeated mice classified as Low or High Submission according to the time spent in Defense/Submission behavior in the first defeat. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$, significant difference with respect to IRSD Low Submission group.

		IRSD low submission	IRSD high submission
Splash test (grooming)	Frequency	25,12 (± 1,09)	20,11 (± 0,98) +++
	Latency	92,75 (± 7,82)	97,46 (± 7,7)
	Time	96,67 (± 5,42)	80,48 (± 4,94) +
Social interaction test	ISI	0,50 (± 0,11)	0,44 (± 0,02) ++
EPM (open arms)	Entries	15,5 (± 1,68)	18,33 (± 1,81)
	Latency	9,83 (± 3,35)	7,96 (± 3,92)
	Time	99,51 (± 9,02)	110,39 (± 10,37)
	Percentage of time	45 (± 3,9)	59,15 (± 3,55) ++
	Percentage of entries	43,36 (± 3,2)	53,57 (± 4,1) +
Hole-board test (dips)	Frequency	28,47 (± 3,69)	32,56 (± 4,69)
Tail suspension test	Time of immobility	8,41 (± 0,65)	8,96 (± 0,54)

spent less time [t (34) = −2.581; p < 0.01; $d = -0.86$] and showed a lower frequency [t (34) = −3.036; p < 0.01; $d = -1.01$] of submission in the fourth episode of defeat. Low submissive mice also received a lower number of threats from opponents in the first [t (34) = −2.267; $p < 0.05$; $d = -0.76$] and fourth [t (34) = −2.351; p < 0.05; $d = -0.78$] episodes of defeat.

Regarding the influence of the submissive profile on the behavioral measures obtained shortly after the last defeat exposure, the Student *t*-test showed that, in comparison to High submissive mice, Low submissive mice performed more grooming behavior [t (34) = 3.44; p < 0.001; $d = 1.15$], spent more time engaged in this behavior [t (34) = −2.212; p < 0.05; $d = 0.74$], had a higher ISI [t (28) = 2.672; p < 0.01; $d = 0.86$] and showed a lower percentage of time [t (34) = 2.685; $p < 0.01$; $d = 0.90$] and percentage of entries [t (34) = 1.964; $p < 0.05$; $d = 0.66$] into the open arms of the EPM (see Table 2).

The submissive profile also had an influence on resilience or vulnerability to the short- and long-term behavioral effects of IRSD (see Fig. 3). When Low or High submissive mice were compared to the non-defeated control mice by means of ANOVA, the frequency of grooming [F (2, 43) = 7.67; $p < 0.001$; $\eta^2 = 0.26$], ISI [F (2, 43) = 9.631; $p < 0.001$; $\eta^2 = 0.31$] and percentage of time spent in the open arms of the EPM [F (2, 43) = 3.966; $p < 0.05$; $\eta^2 = 0.16$] were significant. Only High submissive mice showed a reduction in the frequency of grooming in comparison to the control group ($p < 0.05$; Fig. 3a). Both subgroups of

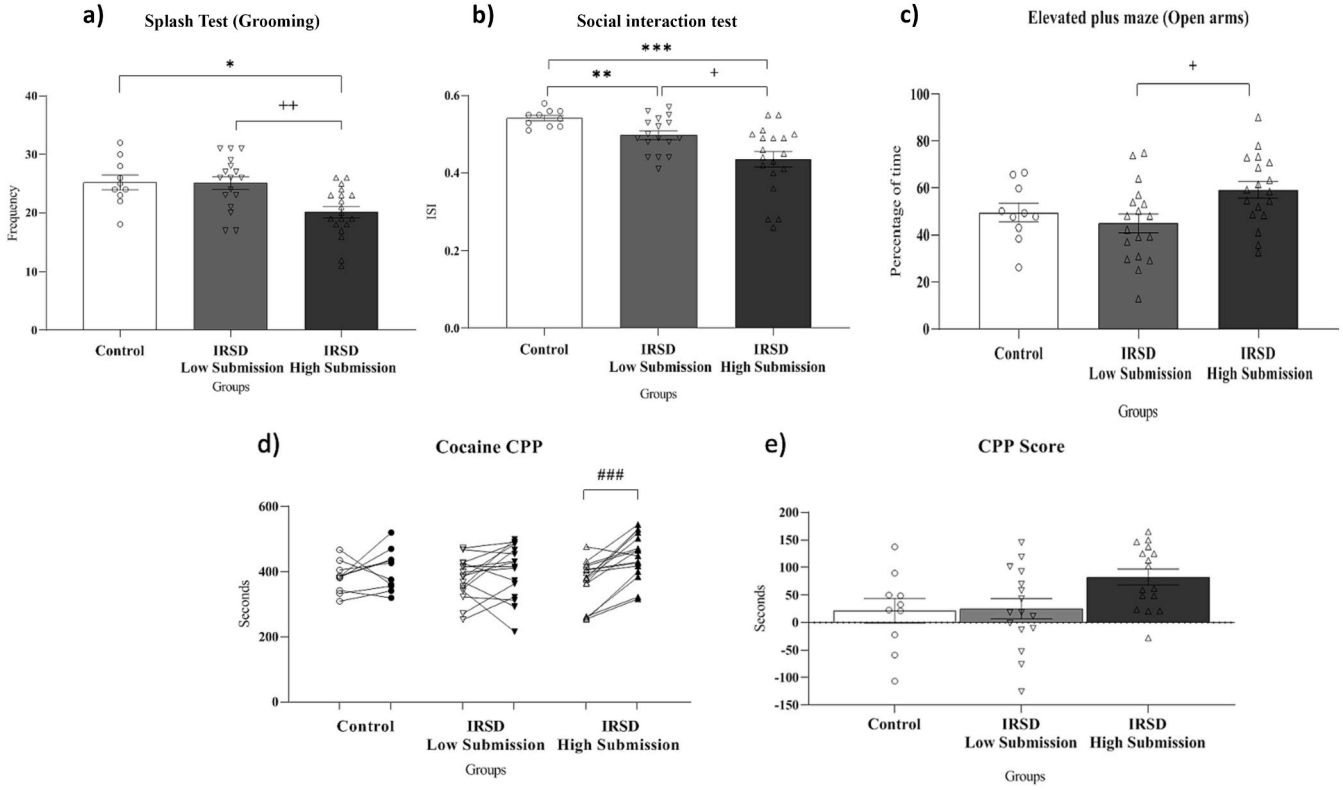

Fig. 3. Behavioral profile during social defeat and resilience to the effects of IRSD. One group of early adolescent male mice was not exposed to stress (CONTROL, $n = 10$) and the other group was exposed to IRSD on PND 27, 30, 33 and 36 ($n = 36$). The behavior of mice during social defeat was evaluated and the group of defeated mice was divided into two subgroups, IRSD Low Submission and IRSD High Submission, according to their scores for time spent in Defense/Submission behavior during the first episode of defeat. a) Bars represent the mean (±SEM) number of times that the mice performed grooming behavior. * p < 0.05, ++ p < 0.01, significant difference with respect to the CONTROL group and IRSD High Submission group, respectively. b) Bars represent the mean (±SEM) ISI. ** p < 0.01, *** $p < 0.001$, significant difference with respect to the CONTROL group; + p < 0.05, significant difference between Low and High Submissive groups. c) Bars represent the mean (±SEM) percentage of TOA on the EPM. + p < 0.05, significant difference between Low and High Submissive groups. d) Effects of IRSD on cocaine-induced CPP according to the behavioral profile of mice in the social defeats. All mice (CONTROL, IRSD Low Submission and IRSD High Submission groups) were conditioned with 1.5 mg/kg of cocaine. Lines represent the time (in seconds) spent in the drug-paired compartment in the pre-conditioning test (Pre—C, empty symbols) and in the post-conditioning test (Post-C, filled symbols). ### $p < 0.001$, significant difference in the time spent in the drug-paired compartment in Post-C vs. Pre-C test. e) CPP score in control and IRSD Low/High Submission subgroups. Bars represent the mean (±SEM) time (in seconds) spent in the drug-paired compartment in Post-C minus that spent in the same compartment in Pre—C.

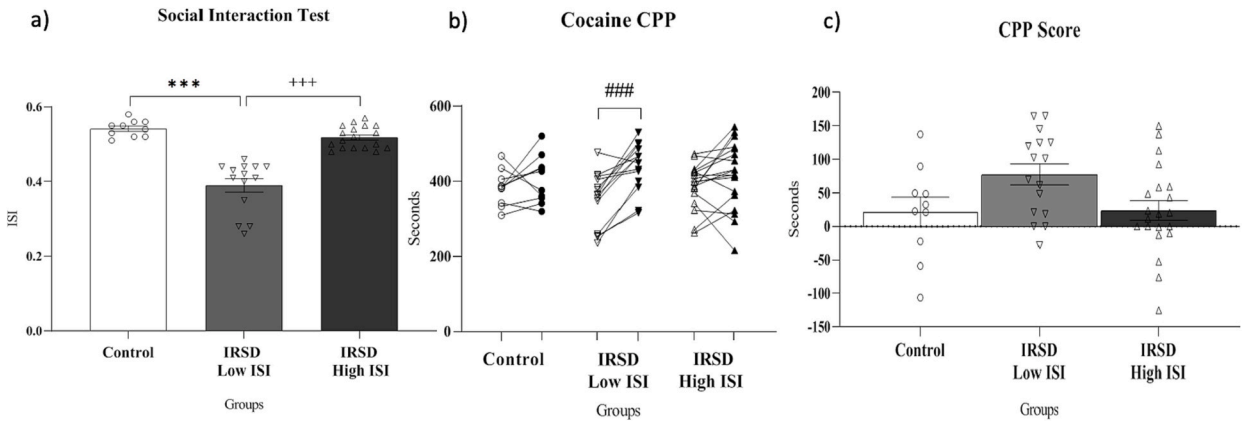

Fig. 4. Behavior in the social interaction test and cocaine reward. One group of early adolescent male mice was not exposed to stress (CONTROL, $n = 10$) and the other group was exposed to IRSD on PND 27, 30, 33 and 36 ($n = 36$). a) Resilience to the short-term effects of IRSD on the social interaction test. The behavior of mice in the social interaction test was evaluated. The group of defeated mice was divided into two subgroups according to their index of social interaction (ISI), IRSD Low Interaction and IRSD High Interaction. Bars represent the mean (±SEM) ISI. *** $p < 0.001$, significant difference with respect to the CONTROL group; +++ $p < 0.001$, significant difference with respect to the IRSD High Interaction group. b) Effects of IRSD on cocaine-induced CPP according to the behavioral profile of defeated mice in the social interaction test. All mice (CONTROL, IRSD Low ISI and IRSD High ISI groups) were conditioned with 1.5 mg/kg of cocaine. Lines represent the time (in seconds) spent in the drug-paired compartment in the pre-conditioning test (Pre—C, empty symbols) and in the post-conditioning test (Post-C, filled symbols). ### $p < 0.001$, significant difference in the time spent in the drug-paired compartment in Post-C vs. Pre-C test. c) CPP score in control and IRSD Low/High ISI subgroups. Bars represent the mean (±SEM) time (in seconds) spent in the drug-paired compartment in Post-C minus that spent in the same compartment in Pre—C.

defeated mice showed a reduction of ISI with respect to the control group, although the significance of the difference with respect to controls was higher in High submissive ($p < 0.001$) than in Low submissive mice ($p < 0.01$; Fig. 3b). In addition, as mentioned before, post-hoc comparison of the ANOVAs showed a difference between Low and High submissive mice in the frequency of grooming ($p < 0.01$; Fig. 3a), ISI ($p < 0.05$; Fig. 3b) and percentage of time in the open arms of the EPM ($p < 0.05$; Fig. 3c).

Regarding the influence of the submissive profile on the long-term effects of IRSD on cocaine reward (see Fig. 3d and e), the ANOVA showed a significant effect of the variable Days [$F(1, 39) = 16.483$; $p < 0.001$; $\eta p^2 = 0.30$] and the interaction Days X Group [$F(2, 39) = 3.860$; $p < 0.05$; $\eta p^2 = 0.17$]. The variable Group was not significant. The Bonferroni test revealed that only the High submissive mice developed CPP (significant difference between Pre- and Post-C, $p < 0.001$).

3.3. Behavioral response in the social interaction test and resilience to the effects of IRSD (cocaine CPP)

In order to evaluate resilience to the effects of IRSD in the social interaction test, defeated mice were separated into two groups according to their index of social interaction (ISI) score (below or above the median of the defeated group, which was 0.48): Low ISI or High ISI. A one-way ANOVA revealed a significant effect of the variable Group [$F(2,39) = 41.420$, $p < 0.001$; $\eta p^2 = 0.68$]. Bonferroni post-hoc comparisons indicated that the Low ISI group was significantly different from the control and High ISI groups (ps < 0.001; Fig. 4a). Thus, the mice in the High ISI defeated group were resilient to the impairing effects of IRSD on social interaction, since they did not engage in less social interaction.

Resilience to the impairing effects of IRSD on social interaction is associated with resilience to the effects of IRSD on cocaine reward. The ANOVA of the CPP data of the control group and the two groups of defeated mice classified in function of their ISI showed that the variable Days [$F(1,39) = 18.541$; $p < 0.001$; $\eta p^2 = 0.32$] and the interaction Days x Group [$F(2,39) = 4.177$; $p < 0.05$; $\eta p^2 = 0.18$] were significant. The variable Group was not significant. Post-hoc comparisons of the interaction revealed that only the Low ISI group displayed CPP (higher time spent in the drug-paired compartment in Post-C than in Pre—C, $p < 0.001$). The control group of mice not exposed to defeat and the group of defeated mice that displayed a higher social interaction (High ISI group) did not develop CPP (see Fig. 4b and c).

3.4. Behavioral response in the splash test and resilience to cocaine CPP

In order to evaluate resilience to the effects of IRSD in the splash test, the defeated mice were divided into two subgroups according to their scores of Frequency of grooming (below or above the median of the defeated group, which was 23 times), Low FG or High FG. One-way ANOVA showed a significant effect of the variable Group [$F(2,39) = 29,036$; $p < 0.001$; $\eta p^2 = 0.60$]. Bonferroni post-hoc comparisons indicated that the Low FG IRSD group differed significantly from the control and High FG IRSD groups (ps < 0.001; Fig. 5a). When defeated mice were divided into two subgroups according to their scores in Time spent grooming (below or above the median of the defeated group, which was 85.7 s), Low TG or High TG, the one-way ANOVA showed a significant effect of the variable Group [$F(2,39) = 24,411$; $p < 0.001$; $\eta p^2 = 0.55$] and Bonferroni post-hoc comparisons indicated that the Low TG IRSD group was significantly different from the control and High TG IRSD groups (ps < 0.001; Fig. 5b). The High FG and High TG groups did not differ from the control group; some defeated mice were resilient to the effects of IRSD on the splash test and did not show a decrease in the frequency of grooming or in the time spent engaged in this behavior.

Resilience to the effects of IRSD on the splash test is associated with resilience to the long-term effects of IRSD on cocaine-induced CPP (Fig. 5c-f). The ANOVA of the CPP data of the control group and the two groups of defeated mice separated in function of their frequency of grooming showed that only the variable Days [$F(1,39) = 14.873$, $p < 0.001$; $\eta p^2 = 0.28$] was significant. Simple Effects of the variable Days showed that the difference between Pre-C and Post-C was significant only in the Low FG group [$F(1,39) = 15.538$, $p < 0.001$; $\eta p^2 = 0.29$] (Fig. 5c). The ANOVA of the CPP data of the control group and the two groups of defeated mice classified in function of the time spent grooming revealed that the variable Days [$F(1,39) = 18.406$, $p < 0.001$; $\eta p^2 = 0.32$] and the interaction Days x Group [$F(1,39) = 4.479$, $p < 0.05$; $\eta p^2 = 0.19$] were significant. The variable Group was not significant. Bonferroni post-hoc comparison of the interaction showed that only the Low TG IRSD group spent more time in the drug-paired compartment in Post-C than in Pre-C ($p < 0.001$). The control (non-defeated mice) and the

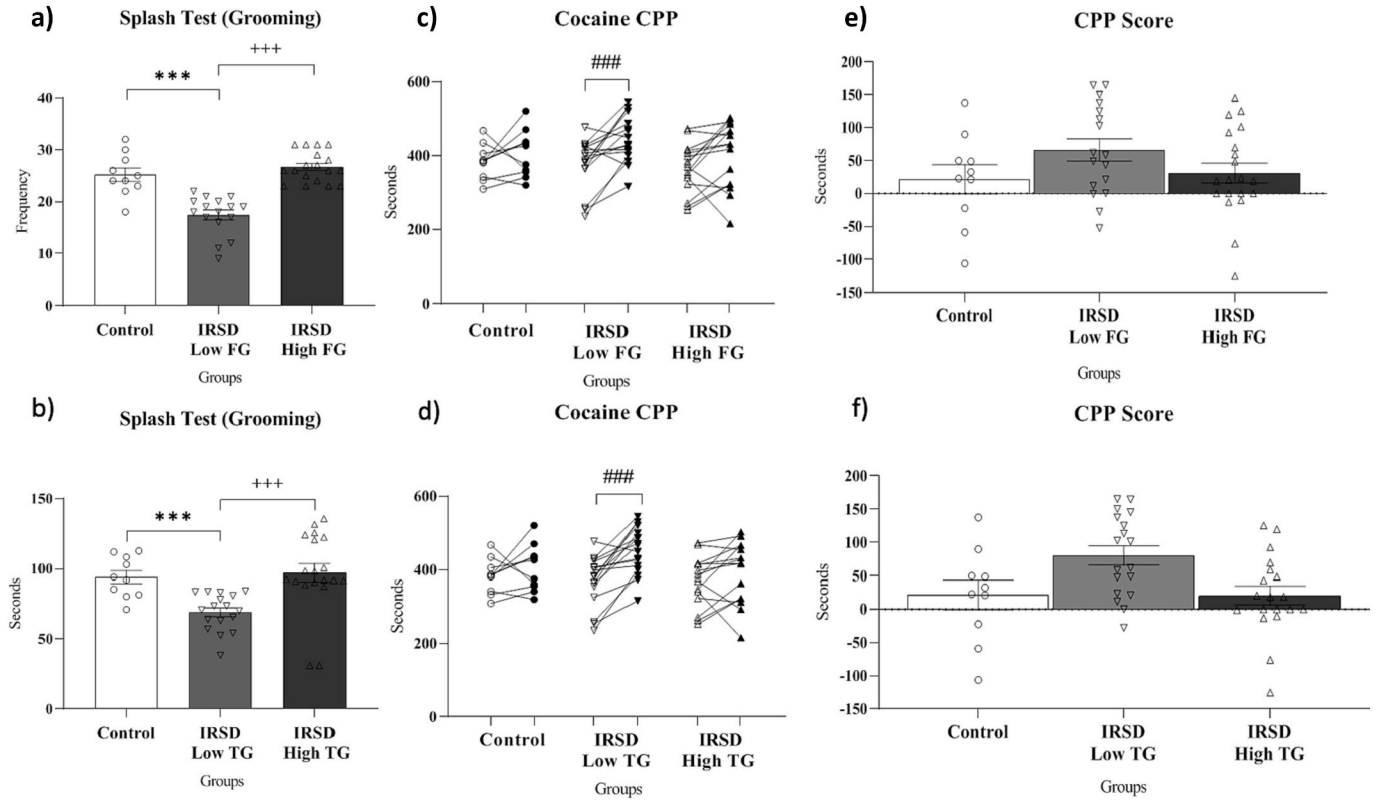

Fig. 5. Behavior in the splash test and cocaine reward. One group of early adolescent male mice was not exposed to stress (CONTROL, $n = 10$) and the other group was exposed to IRSD on PND 27, 30, 33 and 36 ($n = 36$). a) Resilience to the short-term effects of IRSD on the frequency of grooming behavior in the splash test. The group of defeated mice was divided into two subgroups according to their frequency of grooming (FG), IRSD Low FG and IRSD High FG. Bars represent the mean (±SEM) number of times that the mice performed grooming behavior. *** $p < 0.001$; +++ $p < 0.001$ significant difference vs. CONTROL and RSD High FG groups, respectively. b) Resilience to the short-term effects of IRSD on the time spent in grooming behavior in the splash test. The group of defeated mice was divided into two subgroups according to their time spent grooming (TG): IRSD Low TG and High TG. Bars represent the mean (±SEM) time in seconds that the mice spent in grooming behavior. *** $p < 0.001$; +++ $p < 0.001$ significant difference vs. the CONTROL and High TG groups, respectively. c) Effects of IRSD on cocaine-induced CPP according to the behavioral profile of defeated mice in the splash test (frequency of grooming). All mice (CONTROL, IRSD Low FG and IRSD High FG groups) were conditioned with 1.5 mg/kg of cocaine. Lines represent the time (in seconds) spent in the drug-paired compartment in the pre-conditioning test (Pre—C, empty symbols) and in the post-conditioning test (Post-C, filled symbols). ### $p < 0.001$, significant difference in the time spent in the drug-paired compartment in Post-C vs. Pre-C test. d) Effects of IRSD on cocaine-induced CPP according to the behavioral profile of defeated mice in the splash test (time in grooming). Mice (CONTROL, Low TG and High TG groups) were conditioned with cocaine. Lines represent the time (in seconds) spent in the drug-paired compartment in the pre-conditioning test (Pre—C, empty symbols) and in the post-conditioning test (Post-C, filled symbols). ### $p < 0.001$, significant difference in the time spent in the drug-paired compartment in Post-C vs. Pre-C test. e) CPP score in control and IRSD Low/High Frequency of Grooming subgroups. Bars represent the mean (±SEM) time (in seconds) spent in the drug-paired compartment in Post-C minus that spent in the same compartment in Pre—C. f) CPP score in control and IRSD Low/High Time in Grooming subgroups. Bars represent the mean (±SEM) time (in seconds) spent in the drug-paired compartment in Post-C minus that spent in the same compartment in Pre—C.

High TG IRSD groups did not develop CPP (see Fig. 5d). Thus, mice resilient to the effects of IRSD in the splash test were also resilient to the potentiation of cocaine reward induced by IRSD.

3.5. Behavioral response in the elevated plus maze and resilience to cocaine CPP

Although IRSD did not induce effects in the EPM, in order to evaluate whether the behavioral profile of mice in the EPM was influencing resilience to the effects of IRSD on cocaine reward, defeated mice were divided into two subgroups according to their scores of Percentage of time in the open arms (below or above the median of the defeated group, which was 51 %): Low or High %TOA. A one-way ANOVA revealed a significant effect of the variable Group [$F(2,39) = 26.223$; $p < 0.001$; $\eta p^2 = 0.57$]. Bonferroni post-hoc comparisons indicated that the High % TOA ($p < 0.05$) and Low %TOA ($p < 0.01$) groups were significantly different from the control group (Fig. 6a). In addition, the Low and High %TOA groups also differed significantly ($p < 0.001$). Thus, no group of defeated mice displayed a behavioral profile similar to that of control mice.

However, the behavioral profile of mice in the EPM (Low or High % TOA) influenced resilience to the effects of IRSD on cocaine reward (Fig. 6 b-c). The ANOVA of the CPP data for the Control and Low and High %TOA groups showed that only the variable Days [$F(1,39) = 15.283$; $p < 0.001$; $\eta p^2 = 0.28$] was significant. Simple effects of this variable showed that the effect of Days was significant only among the mice that spent a higher percentage of time in the open arms [$F(1,39) = 18.122$; $p < 0.001$; $\eta p^2 = 0.32$]. Thus, the High %TOA spent more time in the drug-paired compartment in Post-C than in Pre—C, while the control and the Low %TOA groups did not develop CPP (see Fig. 6b).

3.6. Behavioral profile in the hole board and tail suspension test did not predict resilience to cocaine CPP

The novelty-seeking (NS) profile of mice (Low vs High NS) and the behavioral profile of mice in the TST (Low or High time spent immobile) did not influence resilience to the effects of IRSD on cocaine reward (see Supplementary Material and Supplementary Figs. 1 and 2).

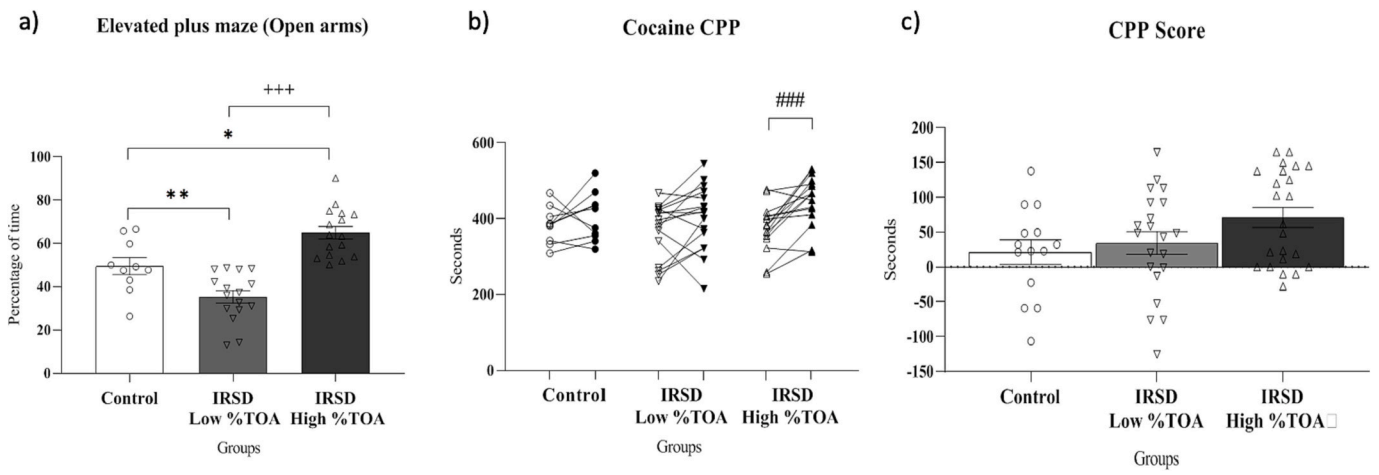

Fig. 6. Behavior in the elevated plus-maze (EPM) and cocaine reward. One group of early adolescent male mice was not exposed to stress (CONTROL, $n = 10$) and the other group was exposed to IRSD on PND 27, 30, 33 and 36 ($n = 36$). a) Short-term effects of IRSD on the EPM. The group of defeated mice was divided into two subgroups according to the percentage of time they spent in the open arms (%TOA) of the EPM, IRSD Low %TOA and IRSD High %TOA. Bars represent the mean (±SEM) percentage of TOA. * $p < 0.05$, ** $p < 0.001$, significant difference vs the control group. +++ $p < 0.001$, significant difference between the High and Low % TOA groups. b) Effects of IRSD on cocaine-induced CPP according to the behavioral profile of defeated mice in the EPM (percentage of time in open arms). All mice (CONTROL, IRSD Low % TOA and IRSD High % TOA groups) were conditioned with 1.5 mg/kg cocaine. Lines represent the time (in seconds) spent in the drug-paired compartment in the pre-conditioning test (Pre—C, empty symbols) and in the post-conditioning test (Post-C, filled symbols). ### $p < 0.001$, significant difference in the time spent in the drug-paired compartment in Post-C vs. Pre-C test. c) CPP score in control and IRSD Low/High %TOA subgroups. Bars represent the mean (±SEM) time (in seconds) spent in the drug-paired compartment in Post-C minus that spent in the same compartment in Pre—C.

4. Discussion

The present study demonstrated that exposure to IRSD is a useful paradigm to study the resilience to the behavioral short- and long-term effects of social stress in adolescent mice. Experience of IRSD during early adolescence induces depression-like symptoms short-term after defeats and increased the rewarding effects of cocaine at the initiation of adulthood, but some mice are resilient to these effects. In particular, mice displaying less defensive/submissive behaviors during episodes of defeat (which can reflect an active coping strategy) are resilient to the effects of social defeat in the splash test and CPP paradigm. In addition, there are several behavioral traits associated to the resilience to the potentiating effect of IRSD on cocaine reward. Mice that are resilient to the depression-like symptoms induced by IRSD in the splash and social interaction tests, as well as mice that spend a lower percentage of time in the open arms of the EPM, are also resilient to the effects of IRSD on cocaine CPP.

4.1. *Effects of IRSD on early adolescent mice*

Exposure to IRSD in early adolescence induces short- and long-term behavioral effects, including the development of depression-like symptoms in adolescent mice and enhanced sensitivity to the conditioned rewarding effects of cocaine in early adulthood. In line with these results, in a previous study in our laboratory we observed that mice exposed to IRSD in late adolescence also displayed a deficit of social interaction (Calpe-López et al., 2020; Calpe-López et al., 2022b) and a decrease in grooming in the splash test (Calpe-López et al., 2020; Calpe-López et al., 2022b and 2022c). However, in the present study, an effect was not observed among mice exposed to IRSD during early adolescence when they performed the EPM (in accordance with the results obtained by Alves-Dos-Santos et al., 2020 with mice exposed to CSDS in early adolescence), or the tail suspension or hole-board tests. In contrast, we have previously observed that mice exposed to IRSD in late adolescence show anxiety-like symptoms in the EPM (Calpe-López et al., 2020; Calpe-López et al., 2022b and 2022c), an elevated stress responsivity in the TST (Calpe-López et al., 2020; Calpe-López et al., 2022c) and a decrease in novelty-seeking in the hole board test (Calpe-López et al., 2022b and 2022c). These results indicate that early adolescent mice experience social defeat less intensely than their older counterparts. In support of this idea, it has been observed that IRSD induces anxiety and cognitive deficits in late (García-Pardo et al., 2015) but not in early adolescent mice (Rodríguez-Arias et al., 2016). Furthermore, early adolescent mice were reported to show less defensive/submissive behavior (Montagud-Romero et al., 2017) and less avoidance/flee behavior (García-Pardo et al., 2015) during the first episode of defeat than late adolescent mice; in addition, aggressive mice confronted with early adolescent mice showed less aggressive behaviors than those confronted with late adolescent mice (García-Pardo et al., 2015; Montagud-Romero et al., 2017). In the same line, levels of corticosterone after social defeat are lower in early than in late adolescent mice (García-Pardo et al., 2015; Montagud-Romero et al., 2017). However, it is important to note that IRSD exposure induces long-term effects on cocaine reward in early adolescent mice, as previously observed in mice exposed to IRSD in late adolescence (Calpe-López et al., 2020; Calpe-López et al., 2022b). This result is also in line with several studies which have demonstrated that mice exposed to IRSD in early adolescence develop a preference for the compartment associated with a low dose of cocaine, which is ineffective in inducing CPP in non-stressed mice (Montagud-Romero et al., 2017; Rodríguez-Arias et al., 2017). Low, non-CPP-inducing doses of cocaine can also be used to test the potentiation of drug reward induced by early life experiences. In this line, mice exposed during PND14–21 to social isolation or social threat were more susceptible to cocaine and acquired CPP with a sub-threshold dose of this drug that failed to induce CPP in unstressed mice (Lo Iacono et al., 2016a and b; Valzania et al., 2017). In previous studies using a higher dose of cocaine we demonstrated that both control and stressed mice acquired CPP, but that it lingered longer in mice exposed to IRSD than in controls during extinction sessions (Rodríguez-Arias et al., 2017), and that only mice exposed to IRSD showed reinstatement of CPP with low priming doses of cocaine (Montagud-Romero et al., 2016). How different doses of cocaine may influence the extent to which resilience/vulnerability persists following IRSD should be the object of future study.

4.2. Resilience to the effects of IRSD is associated with the behavioral profile of mice during episodes of social defeat

Our results also demonstrate that some early adolescence mice are resilient to the effects of IRSD exposure, in accordance with what we have observed in late adolescent mice (Calpe-López et al., 2020). In particular, mice that spent less time in defense/submission behavior during the episodes of defeat were resilient to the short-term depression-like effects induced by IRSD and showed higher social interaction levels and more frequency of grooming in the splash test than mice which spent more time engaged in defense/submission behavior. In fact, regression analyses indicated that the time spent engaged in this behavior in the first episode of defeat predicted the level of social interaction and frequency of grooming (see Suppl. Fig. 3). In addition, there was a negative correlation between the time spent engaged in this behavior in the fourth defeat and the frequency of grooming. Although IRSD did not affect behavior in the EPM, in comparison to high submissive mice, low submissive mice spent a lower percentage of time in the open arms of the EPM, and there was a positive correlation between this measurement and the frequency of defense/submission in the first defeat. In addition, low submissive mice were resilient to the long-term effects of IRSD on cocaine reward and did not develop CPP. In fact, there was a positive correlation between the time spent in defense/submission in the fourth defeat and the CPP score. In line with these results, we have previously observed that late adolescent mice showing low levels of submissive behavior are resilient to the effects of IRSD on cocaine CPP (Calpe-López et al., 2020). Considered together, these results indicate that the maintenance of an active coping strategy (low levels of defense/submission) during episodes of social defeat is a consistent predictor of resilience to the effects of IRSD in adolescent mice. It is important to note that all the experimental mice displayed defeat, given that they all faced a resident mouse with high levels of aggression. As described in the Supplementary material, aggressor/resident mice maintained the motivation and vigor to threaten and attack every intruder in repeated trials of defeat. Thus, the lower level of defense/submission among resilient mice was not a result of differences in the aggressive behavior of the opponent resident mice. In fact, the reduction of threat between the first and fourth episodes of defeat was accompanied by an increase in attack, and was probably due to the behavioral changes observed in experimental mice (an increase in defense/submission and a reduction of avoidance/flee behavior).

4.3. Resilience to the short-term depression-like symptoms of IRSD is associated with resilience to its long-term effects on cocaine reward

Among our experimental animals, there was a subgroup of defeated mice that was resilient to the impairing effects of IRSD on social interaction and that did not engage in less social interaction following social defeat. This result is in line with the observations of other studies with mice exposed to CSDS (Alves-dos-Santos et al., 2020) or to IRSD (Reguilón et al., 2022) during early adolescence. In addition, we observed that resilience to social avoidance is associated with subsequent resilience to the potentiation of cocaine reward, since defeated mice with an ISI similar to that of control mice did not acquire cocaine CPP. In relation to this, it is important to note that there was a quasi-significant negative correlation between ISI and CPP score, which suggests the importance of this variable in modulating the sensitivity of mice to the rewarding effects of cocaine. In line with this, early adolescent rats that were isolated between 5 and 25 days acquired CPP with a dose that was ineffective in socially housed rats (Cuesta et al., 2020; Starosciak et al., 2012; Zakharova et al., 2009). The association between resilience to the effects of defeat on ISI and on cocaine CPP has previously been observed in male mice exposed to IRSD in late adolescence (Ballestín et al., 2021; Calpe-López et al., 2020), indicating that resilience to defeat-induced social avoidance is consistently related with resilience to the long-term effects of IRSD on the sensitivity of mice to cocaine reward. In contrast with our results, Reguilón et al. (2022) have reported that early adolescent mice classified as resilient based on their level of social interaction show a greater sensitivity to the rewarding effects of cocaine and develop CPP, which is the opposite effect to that observed in mice exposed to IRSD in late adolescence (Ballestín et al., 2021; Calpe-López et al., 2020). Indeed, we used an identical IRSD procedure and mice of the same strain, sex and age, so it is plausible that the divergent results are due to differences in the methodology employed in the social interaction test; in particular, the mouse used as an opponent when evaluating social avoidance. While Reguilón et al. (2022) used a mouse of the same strain (C57BL/6 J), we used a mouse of the OF1 strain (as in the defeat episodes). In this context, it was reported that when the target in the social interaction test was a mouse of the C57BL/6 J strain, both susceptible and resilient mice of the same strain spent more time in the interaction zone than when the aggressive opponent was of the CD1 strain, although the social interaction was significantly higher in resilient than in susceptible mice (Han et al., 2014).

We have also observed that some defeated mice remained resilient to the depression-like effects of IRSD; i.e., they did not display a reduction in the frequency of grooming in the splash test. Similar results have been reported by Alves-Dos-Santos et al. (2020), who observed that approximately half of the mice exposed to CSDS in early adolescence were resilient to the decrease in sucrose preference. In the present study, resilience to the short-term effects of IRSD on the frequency of and time spent in grooming predicts subsequent resilience to cocaine reward; only vulnerable mice displaying reduced grooming behavior acquired CPP three weeks after the last episode of defeat. The same results were reported previously in mice exposed to IRSD in late adolescence (Calpe-López et al., 2020).

Although the response of defeated mice in both the social interaction and splash tests was associated with the subsequent resilience or vulnerability to cocaine reward, we did not detect correlations between ISI and the measurements of grooming. This suggests that these behavioral tests measure unrelated behaviors. In accordance with our results, an absence of correlation between social avoidance and the decrease in sucrose preference induced by exposure to CSDS in early adolescent mice has been reported by Alves-Dos-Santos et al. (2020). Similarly, in a previous study, we did not observe correlations between ISI and grooming in mice exposed to IRSD in late adolescence (Calpe-López et al., 2020).

4.4. Behavioral profile of defeated mice in the EPM is associated with resilience to the long-term effects of IRSD on cocaine reward

Although exposure to IRSD in early adolescence did not induce effects in the EPM, we saw that the behavioral profile of defeated mice in this test was related with the subsequent sensitivity to cocaine reward. After segregating the defeated animals into two subpopulations according to the percentage of time spent in the open arms of the EPM, we observed that mice which spent a lower percentage of time in the open arms were resilient to the long-term effects of IRSD and did not acquire cocaine CPP, while defeated mice that spent a higher percentage of time in the open arms displayed enhanced vulnerability to the rewarding effects of cocaine and developed CPP. These results may seem surprising given the close association between anxiety and cocaine use disorders (Vorspan et al., 2015). However, we have also previously observed that mice exposed to IRSD in late adolescence and which spent a lower percentage of time in the open arms of the EPM did not develop CPP (Calpe-López et al., 2020). As we reported in our previous study, the EPM test not only reveals an anxious state; the higher percentage of time spent in the open arms by the mice that developed CPP might indicate a pre-existing impulsive phenotype (Gass et al., 2014) that predisposes them to be more vulnerable to the effects of cocaine. Furthermore, the EPM entails a conflict between two natural tendencies: the motivation to remain in the closed arms (associated with safety) and the motivation to explore the open arms, which could be a potential danger or threat

Table 3
Percentage of mice in each group (vulnerable vs. resilient to the effects of IRSD on cocaine reward; CPP score higher or lower than 50 s, respectively) that displayed the behavioral traits thought to be associated with resilience.

Behavioral traits associated with resilience	Groups	
	Vulnerable mice	Resilient mice
	(CPP score > 50)	(CPP score < 50)
Low Submission	35,29 %	82,35 %
High ISI	35,29 %	70,59 %
High Frequency grooming	35,29 %	64,71 %
High Time Grooming	35,29 %	64,71 %
Lower % TOA	35,29 %	41,18 %

(Ennaceur and Chazot, 2016). Indeed, in a recent study carried out in our laboratory we demonstrated a positive correlation between the percentage of time spent in the open arms and the distance travelled in the EPM (Calpe-López et al., 2022b), thus supporting the idea that a motivation to explore predominates among mice that spend a higher percentage of time in the open arms. Conversely, mice that spent a lower percentage of time in the open arms would prefer to feel safe than to explore. In support of this hypothesis, we observed a negative correlation between immobility in the tail suspension test and the percentage of time spent in open arms. In addition, behavior during the first episode of defeat was also related with this measure. The percentage of time spent in the open arms correlated positively with the frequency of defense/submission, and negatively with avoidance/flee behavior. Thus, we interpret that defeated mice which are resilient to the long-term effects of IRSD on cocaine reward are those that actively avoid the open arms to stay safe from other potential threats after experiencing attack by an opponent.

5. Conclusion

The present study demonstrates that there are several behavioral traits that are associated with vulnerability or resilience to the effects of IRSD in early adolescent mice. However, as indicated by the absence of significant effects in the regression analyses, none of these behavioral traits alone can predict vulnerability or resilience to cocaine reward (see Supp. Figs. 4 and 5). A limitation of our study is the fact that only a small number of mice ($n = 6$) displayed all the behavioral traits thought to be to be associated with resilience to the effects of IRSD on cocaine reward (low submission behavior, high ISI, high frequency and time in grooming and low percentage of TOA). Indeed, some of the mice that displayed said behavioral traits acquired CPP (i.e., they were vulnerable to the effects of IRSD on cocaine reward), while some mice that did not possess these traits did not develop CPP (i.e., they were resilient). Thus, to possess a given behavioral trait (for example, to be less submissive) is not necessarily enough to protect against the effects of IRSD on cocaine reward. However, as can be seen in Table 3, most of the resilient mice exhibited this trait (82 %), and it was more frequent among resilient than among vulnerable mice (35 %). In addition, it is important to note that a high percentage of mice that developed CPP possessed only two or even less of the behavioral traits associated with resilience, while a high percentage of mice that did not develop cocaine CPP possessed 3 or more of these traits (see Fig. 7).

Highly submissive mice were more vulnerable to the effects of social stress, as they were prone to develop depression-like symptoms shortly after defeat and to exhibit a long-term enhanced vulnerability to the rewarding effects of cocaine. Conversely, the mice with an active coping strategy during episodes of social defeat (mainly reflected by low levels of submission/defense behavior) tended to be resilient to the short-term, depression-like effects of IRSD in the social interaction and splash tests and to the long-term effects of IRSD on cocaine reward (the 82 % of mice that did not develop CPP displayed the low submission trait). Furthermore, defeated mice that were resilient to the development of cocaine CPP showed an absence of social avoidance and unaltered levels of grooming more frequently than vulnerable mice (see Table 3). The behavioral profile in the EPM - characterized by a lower percentage of time spent in the open arms - was also related with resilience to the effects of IRSD on cocaine CPP; however, as can be seen in Table 3, the difference between the percentages of vulnerable and resilient mice that possessed this trait is lower. Indeed, all these variables were associated with resilience to the effects of IRSD in late adolescent mice. Conversely, the behavioral profile of mice defeated in early adolescent in the hole board or tail suspension tests was associated with neither resilience nor vulnerability to the long-term effects of IRSD, in contrast with the previously reported influence of these variables on mice exposed to defeat in late adolescence. From a translational view, our results suggest that resilience to the effects of social stress during adolescence is related with the behavioral profile (coping strategy) of individuals during episodes of stress. In this sense, behavioral interventions that increase the pro-active response of adolescents exposed to bullying could enhance their resilience to the negative consequences of this stressful experience and prevent the development of depressive and addictive disorders.

Ethics statement

The animal study was reviewed and approved by Ethics Committee in Experimental Research (Experimentation and Animal Welfare) of the University of Valencia (A1507028485045).

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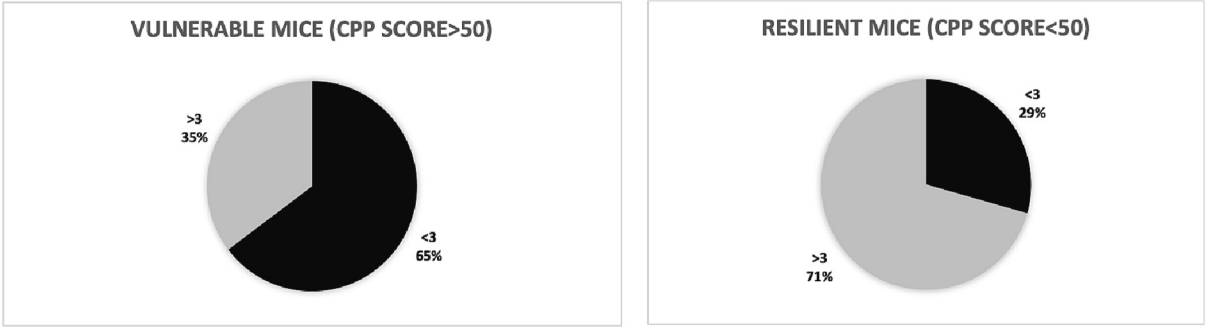

Fig. 7. Percentage of mice in each group (vulnerable vs. resilient to the effects of IRSD on cocaine reward; CPP score higher or lower than 50 s, respectively) that displayed zero, one or two (<3), or three or more (>3) of the following behavioral traits: high percentage of TOA in the EPM; high ISI; high grooming (frequency and time).

Data availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2023.173574.

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