Behavioral and neuroimmune characterization of resilience to social stress: Rewarding effects of cocaine

Caracterización conductual y neuroinmune de la resiliencia al estrés social: Efectos reforzantes de la cocaína

Francisco Ródenas-González*, María del Carmen Blanco-Gandía**, José Miñarro López*, Marta Rodríguez-Arias*.

* Universidad de Valencia. Departamento de Psicobiología. Facultad de Psicología. Avenida Blasco Ibáñez, 21. 46010. Valencia.
** Universidad de Zaragoza. Departamento de Psicología y Sociología. Facultad de Ciencias Sociales y Humanas. C/ Ciudad Escolar s/n, 44003 Teruel.

Abstract

Preclinical studies have shown that social stress increases vulnerability to the reinforcing effects of cocaine. However, the results are not always homogeneous, revealing a subpopulation that does not show a preference for cocaine. Thus, the main aim of the present study was to characterize the behavioral profile of resilient mice to the stress-induced rewarding effects of cocaine using an animal model of repeated social defeat stress (SD). To this end, male adult mice of the C57/BL6 strain were exposed to SD and, three weeks later, assessed using the Conditioned Place Preference paradigm induced by an ineffective dose of cocaine (1mg/kg). Afterwards, the striatal levels of interleukin 6 were measured, as social stress usually induces a neuroinflammatory response. Control mice did not develop CPP, while defeated mice did overall develop a preference for the drug-paired compartment. Based on the conditioning score that they exhibited, the SD sample was subdivided into resilient (did not develop preference) and susceptible mice (developed preference). During the SD sessions, resilient animals showed less flight and submission behaviors than susceptible mice and they presented attack behaviors towards the residents, thereby showing their resistance to being defeated. There were no differences in the neuroinflammatory response, probably due to the long time elapsed after the last SD session. These results suggest that an active coping style to social stress may be decisive in protecting the individual from developing an addiction.

Keywords: Resilience; cocaine; social stress; coping; interleukin 6.

Resumen

Numerosos estudios preclínicos han demostrado que el estrés social incrementa la vulnerabilidad a los efectos reforzantes de la cocaína. Sin embargo, los resultados obtenidos no son homogéneos, observándose siempre una subpoblación que no muestra dicho incremento. Utilizando el modelo de derrota social (DS) repetida en ratones, en este trabajo hemos querido caracterizar conductualmente a los ratones resilientes al incremento de los efectos reforzantes de la cocaína inducido por el estrés social. Utilizamos ratones adultos macho de la cepa C57/BL6 a los que sometimos al protocolo de DS repetida y tres semanas más tarde, realizamos el Condicionamiento de Preferencia de Lugar (CPL) inducido por una dosis no efectiva de cocaína (1mg/kg). Una vez finalizado este procedimiento se midieron los niveles estriatales de interleucina 6, ya que el estrés social produce una respuesta de neuroinflamación. No se observó CPL en los ratones controles, pero los animales derrotados tomados en conjunto desarrollaron preferencia. Sin embargo, esta muestra se pudo dividir en ratones resilientes (no desarrollaron preferencia) y susceptibles (presentaron CPL). Durante las derrotas sociales, los animales resilientes pasaron menos tiempo en las conductas de huida y sumisión que los catalogados como susceptible y presentaron conductas de ataque hacia el ratón residente, manifestando por tanto resistencia a ser derrotados. No se observaron diferencias en la respuesta de neuroinflamación, probablemente debido al largo periodo de tiempo trascurrido desde la última derrota social. Nuestros resultados sugieren que un estilo de afrontamiento activo al estrés social va a ser determinante en la protección del sujeto a desarrollar un trastorno por uso de drogas.

Palabras clave: Resiliencia; cocaína; estrés social; afrontamiento; interleucina 6.

Received: May 2019; Accepted: October 2019.

Send correspondence to: Dra. Marta Rodríguez-Arias.
Dpto. de Psicobiología, Fac. de Psicología, Universitat de València, Av. Blasco Ibáñez, 21, 46010 Valencia-Spain. Tel +34963864637; Fax +34963864668
E-mail: marta.rodriguez@uv.es

Exposure to stress is an environmental factor which has been directly related to the onset of psychiatric disorders such as depression, anxiety or substance abuse disorders. However, not all subjects are equally vulnerable to the consequences of stress (Krishnan et al., 2007; Lutter et al., 2008). Recent years have seen a great increase in the study of the phenomenon of stress resistance. Resilience is defined as the ability of individuals to maintain adaptive psychological and physical functioning, and to avoid the occurrence of mental illness when exposed to chronic or high intensity stress (Charney, 2004), with the mechanisms responsible for resilience promoting an appropriate and non-pathological response to stress (Chmitorz et al., 2018). In recent years, researchers have begun to identify the psychological and biological characteristics of individuals resistant to social stress (Pfau & Russo, 2015). For example, there are a number of behaviors and psychological traits, such as cognitive flexibility, active coping, optimism, or the feeling of belonging to a group, which can favor a resilient response in humans (Wood & Bhatnagar, 2015; Laird , Krause, Funes & Lavretsky, 2019). However, most of these studies have focused on resilience to the development of depression, anxiety or post-traumatic stress disorder (Russo, Murrough, Han, Charney & Nestler, 2012; Krishnan, 2014; Finnell & Wood, 2016), with very few studies assessing resilience to escalating drug use.

Most preclinical studies on stress resilience use the repeated or chronic model of social defeat (SD). This model has great ethological and translational relevance since the most common form of stress experienced by humans originates in their social environment. This model is based on the resident-intruder paradigm, in which a male (intruder) animal is introduced into the territory of another (resident), who will confront and dominate the first (Miczek, Yap & Covington, 2008; Chaouloff, 2013). Numerous studies have shown that repeated SD increases the use of cocaine and alcohol (Miczek et al., 2008; Burke & Miczek, 2014; Rodríguez-Arias et al., 2016, 2017; Montagud-Romero et al., 2016a; Ferrer-Pérez et al., 2018a). This increase has been associated with a neuroinflammatory response since defeated animals have shown an increase in inflammation markers such as cytokines or chemokines, greater blood-brain barrier permeability as well as activation of the microglia (Rodríguez-Arias et al., 2017, 2018; Ferrer-Pérez et al., 2018a).

As with studies on humans, in most preclinical studies the development of resilience to the development of depression or anxiety has been assessed in mice exposed to repeated SD. In these studies, 24 hours after finishing the final SD, animals are categorized as resilient or susceptible depending on their behavior in a social interaction test. Those maintaining higher social contact time are resilient, while the susceptible show social avoidance (Krishnan et al., 2007; Russo et al., 2012; Golden, Covington, Berton & Russo, 2011; Henriques-Alves & Queiroz, 2015; Zhan et al., 2018). Some studies have confirmed that among the factors that mediate resilience is a lower neuroinflammatory response in resilient animals (Wang et al., 2018).

These results have led us to propose as a main objective of the present study the characterization of those mice exposed to repeated SD which are resilient to the long-term increase of the rewarding effects of cocaine. To this end, three weeks after the final SD, we carried out the Conditioned Place Preference paradigm (CPP) with a sub-threshold dose of cocaine, a dose which is not effective in control animals but which does induce preference in those socially defeated (Montagud-Romero et al., 2016a, 2016b). Behavioral characterization was carried out by assessing the behavior of animals showing resilience during SD. Finally, once the behavioral procedure was completed, we studied the neuroinflammatory response by measuring the striatal levels of interleukin 6 (IL6).

Material and Methods

Animals

We used 43 adult male mice of the C57BL6 strain, with 28 as experimental subjects (social defeat) and 15 as a control group (exposed only to exploration). Another 10 male albino mice of the OF1 strain were also used as resident mice in the repeated SD. All mice were purchased from Charles River Laboratories (Barcelona, Spain.). The experimental mice arrived on postnatal day (PND) 21 and were housed in groups of 4 in 26x20x13 cm plastic cages. The 10 OF1 strain mice were housed in isolation for use as residents during repeated SD. The environmental conditions were a temperature of 21 ± 2ºC and a relative humidity of 55%. The mice were kept throughout the procedure in a 12-hour light/dark cycle (8:00-20:00) and with water and pellets *ad libitum,* except during behavioral tests. All procedures for the treatment and care of mice complied with national, regional and local laws and regulations in accordance with international community guidelines, as set out in *European Community Council Directives* (86/609/EEC, 24 *November* 1986). The study was carried out in the Drug Addiction Psychobiology Research Unit of the Department of Psychobiology, Faculty of Psychology, University of Valencia. It was approved by the Animal Experimentation and Welfare Ethics Committee of the University of Valencia 2017/VSC/PEA/00224-A1507028485045.

Pharmacological treatment

The animals were subjected to drug treatment only during the CPP procedure. Mice in both the control and the experimental group were injected intraperitoneally with a 1 mg/kg dose of cocaine dissolved in 0.9% NaCl

solution. This is considered a sub-threshold dose showing no preference of place in the CPP test with standard mice (Maldonado, Rodríguez-Arias, Castillo, Aguilar & Miñarro, 2006; Vidal-Infer, Aguilar, Miñarro & Rodríguez-Arias, 2012), while mice exposed to repeated SD do develop preference (Rodríguez-Arias et al., 2017).

Sample collection

To obtain samples we followed the procedure of previous studies (Ferrer-Pérez et al., 2018b). Mice were killed by cervical dislocation and subsequently decapitated. Brains were quickly removed and the striatum dissected after the procedure described by Heffner et al. (Heffner, Hartman & Seiden, 1980) and kept on dry ice until stored at -80° C.

Before determining IL-6 levels, the brains were homogenized and prepared following the procedure described by Alfonso-Loeches et al. (2010). The striata were homogenized as 250 mg of tissue/0.5 ml of cold lysis buffer (1% NP-40, 20 mM Tris-HCl, pH 8, 130 mM NaCl, 10 mM NaF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 40 mM DTT, 1 mM Na3VO4 and 10 mM PMSF). Brain homogenates were kept on ice for 30 minutes and centrifuged at a speed of 11.519 x g for 15 minutes, after which the supernatant was collected and protein levels were determined by the Bradford assay (Thermo Fisher, ref: 23227).

Experimental design

Table 1 shows the experimental design of the present study in detail. All mice arrived at the laboratory aged 21 days. After three weeks of adaptation in the animal facility, at PND 47, the four SD sessions began. Three weeks after the final SD, we performed the CPP (three days of pre-conditioning, four days of conditioning and one day of post-conditioning). Finally, after completing the entire experimental procedure, the animals were killed to enable the collection of biological samples.

Apparatus and procedure

Social Defeat

The SD protocol carried out in this study has been previously validated and described in detail (Montagud-Romero et al., 2016a; Rodríguez-Arias et al., 2017; Ferrer-Pérez et al., 2019). Repeated SD consists of four 25-minute sessions at 72-hour intervals, on postnatal days 47, 50, 53 and 56. The repeated SD session consists of three phases. In the first phase, the intruder is introduced into the resident's cage for ten minutes, where it is protected from the attacks, but not threat, of the resident by means of a wire partitition. In the second phase, the partition is removed and confrontation is allowed for five minutes. In the third and last phase, the partition is replaced for a further ten minutes.

The repeated SD sessions were recorded with a video camera to enable assessment of the intruder animal's flight, submission and attack behaviors, and the resident's threat and attack behaviors. In the repeated SD with the 15 control mice, a procedure similar to that described above was used, but without the presence of the resident mouse. After completing the paradigm, the analysis of the encounters was carried out using a computer program with which the time spent performing different behaviors can be recorded (Martínez, Miñarro & Simón, 1991).

Conditioned Place Preference (CPP)

CPP is a model based on classical or Pavlovian learning to assess the conditioned reward induced by different stimuli (Bardo & Bevin, 2000; Tzschentke, 2007). It has been widely used to study the reward effects of conditioned addictive drugs (Aguilar, Rodríguez-Arias & Miñarro, 2009; Yap et al., 2015; Rodríguez-Arias et al., 2016; Blanco-Gandía et al. 2017) since contextual stimuli can acquire secondary appetitive properties when combined with a primary enhancer (Tzschentke, 2007).

For CPP, we used 12 identical plexiglas cages with two compartments of equal size (30.7 cm long by 31.5 cm wide by 34.5 cm high), separated by a central gray area (13.8 cm long by 31.5 cm wide by 34.5 cm high). The compartments have different color walls (white vs black) and different floor texture (smooth in the black compartment and rough for the white). Animals are trained to associate one specific environment with the effect of the drug administered, and the other compartment with saline solution (García-Pardo, Rodríguez-Arias, Miñarro & Aguilar, 2017). A guillotine door separates each compartment from the central compartment. Each of the conditioning compartments has four photoelectric cells, while the central zone has six, to allow the position of the animal and the crossings from one compartment to the other to be recorded. The equipment is controlled by two IBM PC computers running MONPRE 2z software (CIBERTEC, SA, España).

CPP comprises three phases, carried out during the dark cycle and following an 'unbiased' procedure in terms of the spontaneous initial preference (Manzanedo, Aguilar,

Table 1. *Experimental Design.*

	Social Defeat/Exploration					CPP (1mg/kg cocaine)			Sample collection
	1th	2th	3th	4th	3 weeks	Pre-C test	Conditioning	Post-C test	
PND	47	50	53	56		76 - 78	79 - 82	83	84

Rodríguez-Arias & Miñarro, 2001). During the first phase or pre-conditioning (Pre-C), the mice had free access to both compartments of the apparatus for 15 minutes (900s) each day for 3 days. On the third day, the time each animal spent in each compartment was recorded for 900s. Animals showing strong aversion (less than 33% of the session time) or strong preference (more than 67%) for any compartment were excluded from the procedure. In the present experiment, a total of two animals were excluded for not meeting the established criteria. Compartment allocation was counterbalanced. One of the compartments was chosen for association with cocaine in such a way that, within each group, half the animals received the cocaine in the least preferred place and the other half in the most preferred, and compartment color was also balanced. There should be no significant differences in the time that animals spend in the compartment associated with the drug or vehicle in the pre-conditioning phase. This measure is of great importance for the experimental procedure as it helps to avoid any preference bias before starting the experiment.

In the second phase (conditioning), the animals were conditioned with 1 mg/kg of cocaine through four associations with the compartment allocated after Pre-C. It has been observed that 1 mg/kg is a sub-threshold dose, i.e., a dose that does not lead to the acquisition of CPP, unless other variables such as stress or behavioral traits are manipulated (Vidal-Infer et al., 2012; Arenas et al., 2014; Montagud-Romero et al., 2014; Rodríguez-Arias et al., 2016; Blanco-Gandía, Montagud-Romero, Aguilar, Rodríguez-Arias & Miñarro, 2018). The animals received two injections (cocaine and vehicle) each day: a saline administration before being confined to the non-associated compartment for 30 minutes, and after an interval of four hours they received cocaine before being confined to the compartment associated with the drug for 30 min. The central area was not used during conditioning and access to it was blocked by guillotine doors.

During the third phase, post-conditioning (Post-C), on the 8th day of the procedure, the guillotine doors separating both compartments were removed and the time that the mice spent in each compartment, without any treatment, was recorded for 900s. The difference in seconds between the time that the animals remained in the compartment associated with the drug during the Post-C test and the time they spent during the Pre-C test is a measure of the degree of conditioning induced by the drug (*Conditioning Score*). If this difference is positive, then the drug has induced a preference for the drug-paired compartment, while the opposite indicates the induction of an aversion. Once CPP was completed, the defeated animals were divided into resilient or susceptible. Those who exhibited no increase in preference for the cocaine-associated compartment were considered resilient and those whose preference did increase were susceptible.

ELISA IL-6 assay

To determine the concentration of IL-6 in the striatum, we use a mouse IL-6 ELISA kit from Abcam (Ref: ab100712) and followed the manufacturer's instructions. To determine absorbance, we use an iMark microplate reader (Bio-RAD) controlled by Microplate Manager 6.2 software. The optical density was read at 450 nm and the final results were calculated using a standard curve, expressed as pg/mg for tissue samples. The sensitivity of the test is <2 pg/mg. All samples were analyzed in duplicate.

Data analysis

To confirm the effect of repeated SD on CPP, univariate ANOVA was performed on the *Conditioning Score* data with the inter-subject variable 'Stress' with two levels: Exploration and Social Defeat. A K-means cluster analysis was performed using the *Conditioning Score* values to separate the animals into Resilient and Susceptible subgroups. After this division, we performed a new univariate analysis with the three level inter-subject variable 'Group' (Exploration, SD Susceptible and SD Resilient). The same analysis was applied to the striatal levels of IL-6 data. The results obtained in the ethological analysis of SD were analyzed using a two-way ANOVA with the two level inter-subject variable 'Group' (Resilient and Susceptible), and the intra-subject variable 'Defeat' of 2 levels: SD1 (first session) and SD4 (fourth session of social defeat). Post-hoc analyses were performed using the Bonferroni fit test, taking $p < 0.05$, $p < 0.01$ and $p < 0.001$ as significance intervals. The Pearson correlation coefficient was also calculated to determine possible relationships between the Flight variable and *Conditioning Score* of all animals performing repeated SD.

Results

Only susceptible animals develop CPP

Regarding the CPP *Conditioning* Score (Figure 1a), the ANOVA showed a significant effect for the Stress variable [$F(1,36) = 7.147$; $p < 0.05$], indicating that defeated animals spent significantly more time in the drug-associated compartment than non-stressed animals ($p < 0.05$).

Animals were classified as Resilient and Susceptible using K-means cluster analysis [$F(1,24) = 37.748$; $p < 0.001$]. When the group of defeated animals was divided into Resilient and Susceptible subgroups (Figure 1b), ANOVA showed a significant effect for the Group variable [$F(2,38) = 23.289$; $p < 0.001$]. Susceptible animals spent significantly more time in the drug-associated compartment compared to the other two groups ($p < 0.001$ in both cases). Figure 1c shows the individual scores of the three experimental

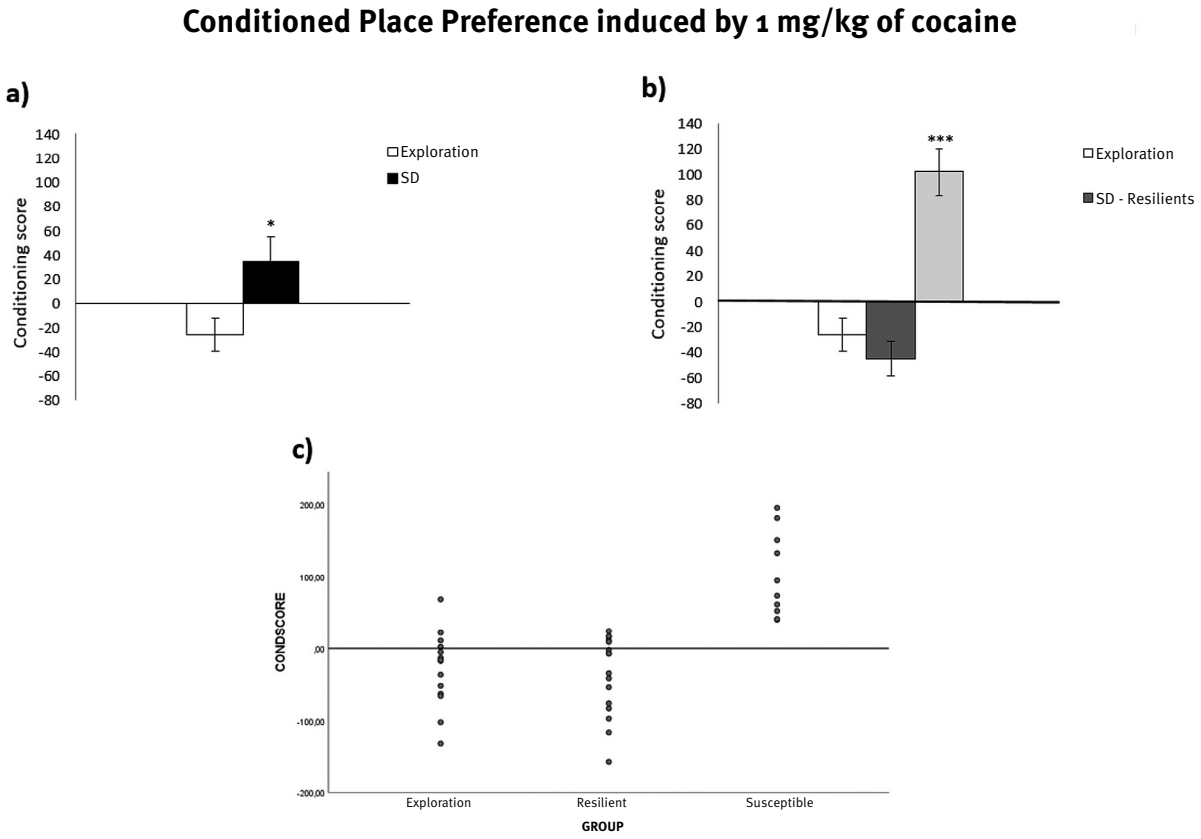

Figure 1. Effect of repeated SD on CPP acquisition induced by 1 mg / kg of cocaine in male C57/BL6 mice. The bars represent the difference in time (s) spent in the compartment associated with the drug before and after the conditioning sessions *(conditioning score)*. (a) Treatment groups: Exploration and repeated SD. (b) After Post-C, the defeated animals were divided into Resilient and Susceptible subgroups according to their level of conditioning. * p<0.05, significant difference compared to Exploration group. *** p <0.001, significant difference compared to Exploration and SD-Resilient group. (c) Individual values of the *Conditioning Score* of the Exploration, Resilient and Susceptible groups.

groups performed by a simple distribution of the *Conditioning Score* data.

Resilient mice show a coping response to stress during SD

Table 2 shows the data relating to the behavior of the defeated mice during the first and fourth SD. In terms of Flight, ANOVA showed a significant effect for the Group variable [$F(1,24) = 16.578$; $p < 0.001$] given the significantly less time SD-Resilient group animals spent behaving in this way ($p < 0.001$). With respect to Submission, ANOVA showed a significant effect for the interaction of Defeat x Stress variables [$F(1,24) = 4.163$; $p < 0.05$], with resilient animals spending less time behaving submissively during the first repeated SD session in comparison to susceptible animals ($p<0.05$).

The presence of attack behavior by intruders against residents was also assessed. We only observed a trend in

Table 2. *Results of repeated SD on intruders.*

Resilient	Flight	Lat. Flight	Submission	Lat. submission	Attack	Lat. attack
SD1	32 ± 3***	15 ± 10	22 ± 6*	56 ± 6	3 ± 2	228 ± 33
SD4	32 ± 3***	3 ± 3	26 ± 7	89 ± 7	0 ± 0	300 ± 0
Susceptible	**Flight**	**Lat. Flight**	**Submission**	**Lat. submission**	**Attack**	**Lat. attack**
SD1	47 ± 7	6 ± 3	34 ± 8+	51 ± 8	1 ± 1	271 ± 30
SD4	51 ± 8	4 ± 8	12 ± 4	99 ± 4	0 ± 0	300 ± 0

Note. Behavior assessed during SD. Data presented as mean values in seconds ± SEM. *p<0.05, ***p<0.001 differences with respect to Susceptibles. + p<0.05 differences with respect to SD4 (group-defeat effect).

the variable Defeat [F(1,24) = 3.023; p = 0.095], which tells us that intruders attacked more in the first SD session. We observed that, compared to 10% of Susceptibles, 25% of the animals classified as Resilient attacked their resident in the first repeated SD. No intruder animals attacked in the fourth repeated SD.

Finally, we assessed the relationship between the Flight behavior shown by all intruders by adding the first and fourth meeting of the repeated SD and their *Conditioning Score* in the CPP to see if the time spent behaving in these ways could be an indicator of conditioning which would occur later (Figure 2). A significant Pearson correlation coefficient was only obtained between the time in Flight mode and the *Conditioning Score* (r = 0.241, p <0.05). That is to say, the longer flight behavior continued during the

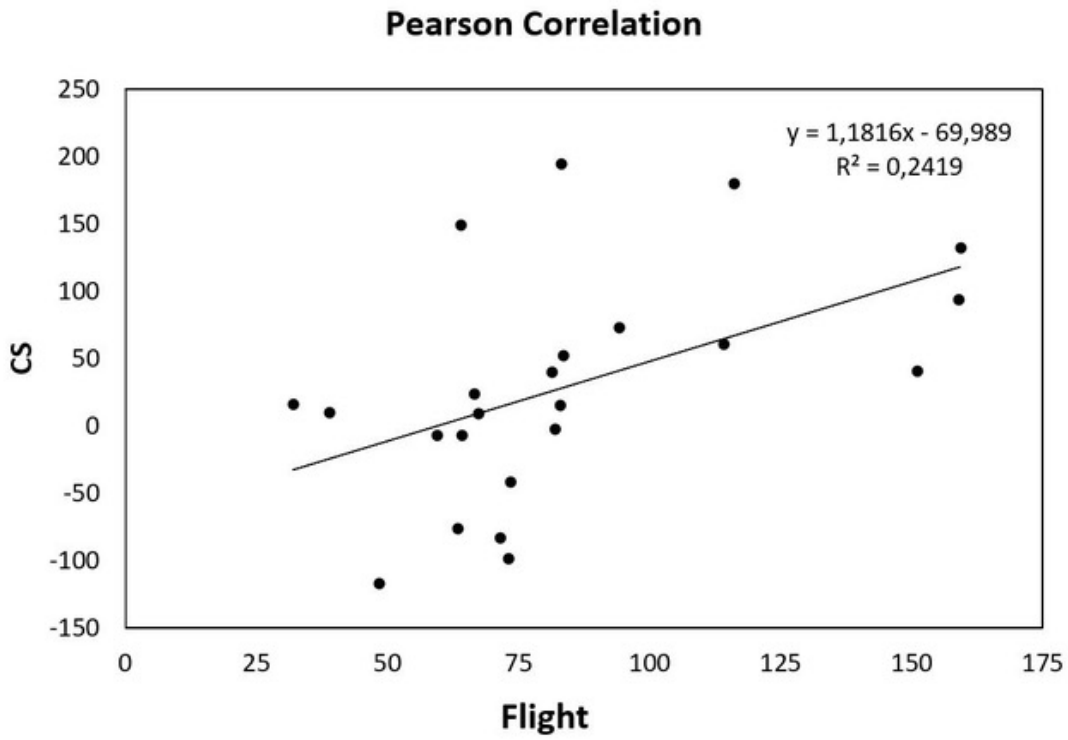

Figure 2. Regression plot for the Pearson correlation between flight during repeated SD and *Conditioning Score* (CS). The trend line represents the linear regression of data (y = 1.1816x - 58.989; r2 = 0.2419).

SD encounters, the greater the preference for the drug in CPP.

Regarding Threat by residents (Table 3), the ANOVA yields an effect for Defeat [F(1,24) = 6.535; p <0.05], indicating that residents threatened more in SD1 than in

Table 3. *Results of repeated SD on residents.*

Resident vs Resilient animals	Threat	Lat. threat	Attack	Lat. attack
SD1	36 ± 5#	10 ± 4	26 ± 5	4 ± 15
SD4	28 ± 5	4 ± 1	22 ± 3	4 ± 1
Resident vs Susceptible animals	**Threat**	**Lat. threat**	**Attack**	**Lat. attack**
SD1	36 ± 7#	6 ± 2	29 ± 9	37 ± 29
SD4	21 ± 4	8 ± 3	33 ± 4	3 ± 1

Note. Social interaction of Residents during the intruder-resident paradigm to induce SD. Data presented as mean values in seconds ± SEM. Differentiation between Residents attacking what were later categorized as Resilients and Susceptibles. # p<0.05 with respect to SD4 (defeat effect).

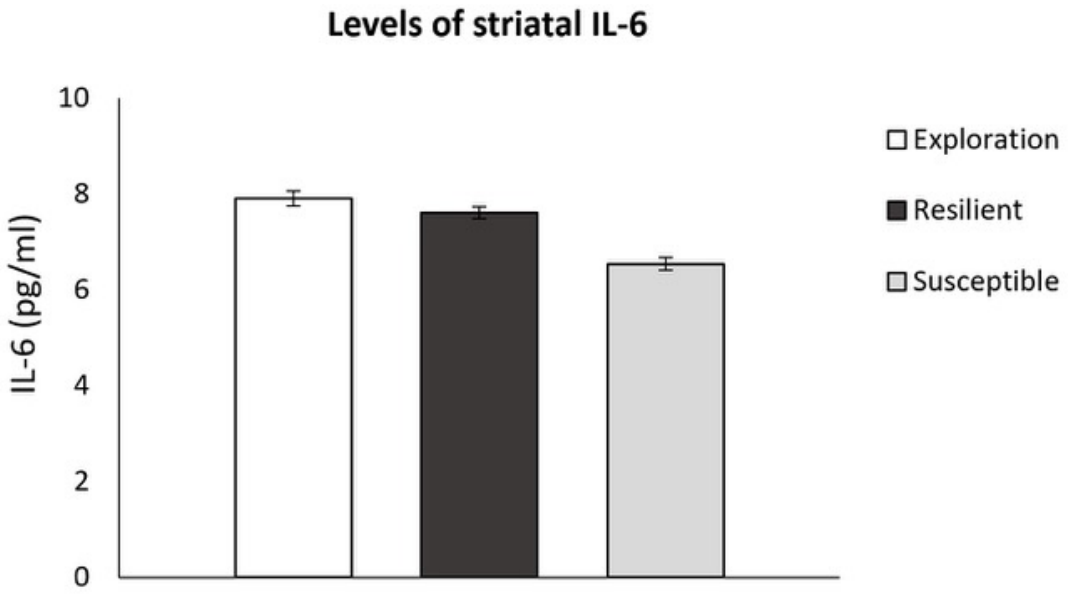

Figure 3. Striatal levels of IL-6. Effect of repeated SD on IL-6 levels in male C57/BL6 mice, taking into account the subdivision into resilient and susceptible. Data are shown as means ± S.E.M. (pg/ml).

SD4 ($p < 0.05$). However, there are no significant differences in the Group variable, implying that both Resilient and Susceptible were exposed to the same stress.

Striatal levels of Il6

The ANOVA for striatal levels of IL-6 (Figure 3) yielded no significant differences.

Discussion

The results of the present study confirm that repeated SD increases the rewarding effects of cocaine in CPP, but we have also demonstrated for the first time that the results obtained in stressed animals are not homogeneous. In defeated animals we can distinguish a susceptible population which has developed CPP with a non-effective dose of cocaine. However, there are also some defeated animals which behave like unstressed animals, that is, they are resilient without not developing CPP, although perhaps the most interesting result is that coping with SD is different in both types of animals. Resilients exhibit lower levels of flight and submissive behavior when facing the aggressor during SD. Flight behavior correlates positively with the analyzed results of CPP, i.e., the stronger the flight behavior, the more the animal will develop a preference for cocaine. Therefore, an active coping response, with less flight and submission during a social stressor, reduces sensitization to the rewarding effects of cocaine. These resilient mice also show attack behaviors against the resident, manifesting resistance to defeat, something that is not observed in any of the susceptible animals. The changes in IL-6 levels do not differ between stressed or control animals, and no difference is observed between those that are resilient or susceptible. This may be because our study was carried out three weeks after the final SD.

Resilience and susceptibility to increased cocaine reinforcing effects

Our results confirm that the experience of repeated SD during adulthood induces a long-term increase in the conditioned rewarding effects of a sub-threshold dose of cocaine (1 mg/kg) since we assessed this three weeks after the final repeated SD. The CPP paradigm is widely used to assess the conditioned effects of drugs (Aguilar et al., 2009) and reflects their secondary motivational properties, as well as their potential for abuse (Tzschentke, 2007). Exposure to repeated SD may thus induce a long-term increase in the motivational value of cocaine, thereby increasing its potential for abuse in stressed subjects. Our results confirm numerous studies showing that SD in adolescent and adult mice increases the rewarding effects of cocaine using CPP (Arenas et al., 2016; Montagud-Romero et al., 2016a; Rodríguez-Arias et al., 2015, 2017; Ferrer-Pérez et al., 2018a), or self-administration of cocaine (Boyson, Miguel, Quadros, DeBold & Miczek, 2011; Holly et al., 2016; Newman, Leonard, Arena, Almeida & Miczek, 2018; Arena, Covington, Herbert, DeBold & Miczek, 2019).

This study actually goes further and shows that although as a whole in our population of defeated mice they all develop preference with a sub-threshold dose of cocaine, we can distinguish two types of subjects. Resilient mice, despite being stressed, do not respond to the conditioned

rewarding effects of cocaine (CPP). Conversely, susceptible animals do develop increased preference for the cocaine-associated compartment. Although there is a great deal of evidence linking stress to the development of addictive behaviors (Lüthi & Lüscher, 2014; Polter & Kauer, 2014; Gold, Machado-Vieira & Pavlatou, 2015), it has also been shown that there are subjects who develop good psychosocial competence in high-risk conditions such as child abuse or adverse socioeconomic status (McGloin & Widom, 2001; Hjemdal, Friborg & Stiles, 2012; Brody et al., 2013). However, there are practically no studies with animal models assessing the phenomenon of resilience to the development of vulnerability to drug use after exposure to a social stressor. A recent study using exposure to the smell of a predator as a stress model classified its mice as resilient and susceptible based on the presence of anxiety in the cruciform raised labyrinth and avoidance of the context associated with the smell (Brodnik, Double, España & Jaskiw, 2017). This study observed that susceptible mice showed increased motor and dopaminergic effects of cocaine as well as a greater motivation to self-administer this drug. These effects were not seen in resilient animals, although in both types of mice an increase in cocaine-induced DA release was observed.

Different coping with social stress in resilient and susceptible animals

Repeated SD is a naturalistic model of social stress which mimics real-life situations and therefore has great ecological and ethological validity (Tornatzky & Miczek, 1993). Some recent research, using animal models of social stress, have observed that coping strategies are associated with resilience or vulnerability to stress (Wood et al., 2015; Chen et al. 2015, Finnell et al., 2017; Pearson-Leary et al., 2017). However, these studies classify animals as resilient or susceptible based on social behavior and anxiety shown by animals on the day after the final SD (Russo et al., 2012; Krishnan, 2014; Finnell & Wood, 2016). In these studies, resilient mice do not present anhedonia (Delgado et al., 2011), social avoidance (Krishnan et al., 2007; Golden et al., 2011; Henriques-Alves & Queiroz, 2015) or avoidance at the smell of a predator (Brodnik et al., 2017). To date, no studies have characterized animals resilient to the increased rewarding effects of drugs of abuse and, therefore, it is not known whether different stress coping strategies influence the sensitivity to such rewarding effects. What we do know is that mice showing no anxiety behavior or avoidance at the smell of a predator have neurochemical adaptations that specifically affect the function of the DA system and could therefore modify the rewarding efficacy of cocaine (Brodnik et al., 2017).

The ethological study of behavior during the social defeats showed firstly that there were no differences in the behavior of the resident animals towards intruders, whether resilient or susceptible. That is, all were exposed to the same level of stress. However, we did observe that the mice which would later be classified as resilient exhibited less flight behavior compared to the susceptible mice. In addition, we observed a positive correlation between flight behavior and the increase in the conditioned rewarding effects of cocaine in the CPP. The less the animals flee, the lower the rewarding effect produced by cocaine. Likewise, resilient animals also showed less submissive behavior during the first SD, although we no longer observed differences between resilient and susceptible animals in the fourth SD. Resilient mice, experiencing that their coping behaviors do not reduce the intensity of the attack, exhibit a behavioral adaptation. The flexibility of coping strategies has been associated with indicators of emotional resilience, such as reduced HHA axis reactivity and increased neuroplasticity (Hawley et al., 2010, Lambert et al., 2014). Our results therefore indicate that active coping and adequate adaptation reduce the rewarding effects of cocaine. Supporting our results, other studies have also confirmed that mice which do not have passive coping strategies such as flight show less anhedonia (Wood et al., 2015), less anxiety and greater social interaction (Duclot, Hollis, Darcy & Kabbaj, 2011; Hollis, Duclot, Gunjan & Kabbaj, 2011; Kumar et al., 2014). Resilient animals also exhibited attack behaviors against residents during the first confrontation, this active coping strategy having been associated with defeat resistance (Finnell & Wood, 2016).

In short, resilient animals develop an active stress coping strategy, since they attack the resident and take longer to accept they have been defeated. This resistance can cause the resilient to experience SD less intensely than the susceptible animals do. It has been observed that mice employing active coping behaviors during SD evidence lower plasma corticosterone levels, greater capacity for noradrenergic response during stress and greater sympathetic activity in response to defeat (Wood, Walker, Valentino & Bhatnagar, 2010; Gómez-Lázaro et al., 2011; Pérez-Tejada et al., 2013). This type of response is very adaptive, since it allows the response to stress to be limited (Koolhaas et al., 2011). Another factor which can explain the development of resilience is the feeling of control during SD, since the resilient mice do not flee from the aggressor and even exhibit attack behaviors. Interestingly, cocaine use is only increased in intruder mice, but not in residents which initiate the attack, although in both types of animals there is a hormonal response to stress (Covington & Miczek, 2001, 2005; Covington et al., 2005; Boyson et al., 2014). The resident mouse maintains control of the encounter, which may exert a protective effect on the stress response of the hypothalamus-pituitary-adrenal axis (Boyson et al., 2014). Therefore, our resilient animals may experience a certain level of control of the stress situation.

Conversely, the susceptible animals showed passive confrontation, accepting defeat with more time in flight and submission and without presenting any aggressive behavior towards the resident. This passive coping during SD has previously been associated with the onset of anxiety and depression (Wood et al., 2010, Chen et al., 2015, Pearson-Leary et al., 2017).

Neuroinflammation response after repeated SD

In the 1990s, the so-called neuroinflammatory theory of depression was proposed (for example, Maes et al., 2009), based on the increase in inflammatory mediators in patients with depression. There are currently numerous studies demonstrating the role of the immune system in the vulnerability to the development of mental illness (Réus et al., 2015; Menard, Pfau, Hodes & Russo, 2017). It is also believed that substance use disorder is related to changes in the activity of the immune system (Clark, Wiley & Bradberry, 2013; Cui, Shurtleff & Harris, 2014). Both clinical and preclinical studies have shown that psychostimulants such as cocaine activate central and peripheral components of the immune system (Clark et al., 2013; Araos et al., 2015; Moreira et al., 2016). More recently it has also been shown that social stress triggers an activation of the immune system, increasing peripheral levels of cytokines, activating microglia or even increasing the permeability of the blood brain barrier (Pfau & Russo, 2016; Rodríguez-Arias et al., 2017, 2018; Ferrer-Pérez et al., 2018a).

It has described that after SD, susceptible mice which develop social isolation and anxiety show higher levels of IL-6 than resilient animals (Hodes, Ménard & Russo, 2016). However, our results do not confirm this lower inflammatory response in resilient animals. Il-6 levels were not higher in defeated animals compared to controls, and no differences were observed between resilient and susceptible mice. The discrepancy in the results may be mainly due to the fact that in the study by Hodes et al. (2016), the measurement of IL-6 was carried out 24 hours after the final SD, while in our study it was done at the end of the entire procedure, when more than a month had passed since the final repeated SD. Similarly, we had previously demonstrated that after CPP, increases in striatal levels of IL-6 in defeated animals were no longer observed (Ferrer-Pérez et al., 2018a). Since the characterization of animals as resilient or susceptible requires the development of CPP, our experimental design involves making measurements at least four weeks after the final SD. Our results therefore indicate that one month after the final SD there are no differences in the neuroinflammatory response.

Animal models are a very powerful tool, but we must be cautious when transferring the results to human behavior. We can extrapolate from the SD model to situations of psychological or social stress to which we are exposed for much of our lives. Our results allow the identification of some behavioral characteristics which appear in animals resistant to this SD and which can act as a protective factor against the development of drug addiction. Active but flexible coping stands out as the most relevant behavioral characteristic of resilient subjects. The study of behavioral or pharmacological strategies underlying resilience will allow us to reduce vulnerability to SUD induced by social stress.

Acknowledgements

Ministerio de Ciencia, Innovación y Universidades (MICINN), PSI2017-83023.

Instituto de Salud Carlos III, Red de Trastornos Adictivos (RD16/0017/0007) and European Union, ERDF Funds "a way to build Europe".

Conflict of interests

The authors declare no conflict of interest.

References

Aguilar, M. A., Rodríguez-Arias, M. & Miñarro, J. (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Research Reviews, 59*, 253-277. doi:10.1016/j.brainresrev.2008.08.002.

Alfonso-Loeches, S., Pascual-Lucas, M., Blanco, A. M., Sanchez-Vera, I. & Guerri, C. (2010). Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. *Journal of Neuroscience, 30,* 8285-8295. doi:10.1523/JNEUROSCI.0976-10.2010.

Araos, P., Pedraz, M., Serrano, A., Lucena, M., Barrios, V., García-Marchena, N.,... Rodríguez de Fonseca, F. (2015). Plasma profile of pro-inflammatory cytokines and chemokines in cocaine users under outpatient treatment: Influence of cocaine symptom severity and psychiatric co-morbidity. *Addiction Biology, 20,* 756-772. doi:10.1111/adb.12156.

Arena, D. T., Covington, 3., Herbert, E., DeBold, J. F. & Miczek, K. A. (2019). Persistent increase of I.V. cocaine self-administration in a subgroup of C57BL/6J male mice after social defeat stress. *Psychopharmacology, 236*, 1-11. doi:10.1007/s00213-019-05191-6.

Arenas, M. C., Aguilar, M. A., Montagud-Romero, S., Mateos-García, A., Navarro-Francés, C. I., Miñarro, J. & Rodríguez-Arias, M. (2016). Influence of the novelty-seeking endophenotype on the rewarding effects of psychostimulant drugs in animal models. *Current Neuropharmacology, 14*, 87-100. doi:10.2174/1570159X13666150921112841.

Arenas, M. C., Daza-Losada, M., Vidal-Infer, A., Aguilar, M. A., Miñarro, J. & Rodríguez-Arias, M. (2014). Capacity of novelty-induced locomotor activity and the hole-board

test to predict sensitivity to the conditioned rewarding effects of cocaine. *Physiology & Behavior, 133*, 152-160. doi:10.1016/j.physbeh.2014.05.028.

Bardo, M. T. & Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward? *Psychopharmacology, 153*, 31-43. doi:10.1007/s002130000569.

Blanco-Gandía, M. C., Cantacorps, L., Aracil-Fernández, A., Montagud-Romero, S., Aguilar, M. A., Manzanares, J.,... Rodríguez-Arias, M. (2017). Effects of bingeing on fat during adolescence on the reinforcing effects of cocaine in adult male mice. *Neuropharmacology, 113,* 31-44. doi:10.1016/j.neuropharm.2016.09.020.

Blanco-Gandía, M. C., Montagud-Romero, S., Aguilar, M. A., Miñarro, J. & Rodríguez-Arias, M. (2018). Housing conditions modulate the reinforcing properties of cocaine in adolescent mice that binge on fat. *Physiology & Behavior, 183*, 18-26. doi:10.1016/j.physbeh.2017.10.014.

Boyson, C. O., Holly, E. N., Shimamoto, A., Albrechet-Souza, L., Weiner, L. A., DeBold, J. F. & Miczek, K. A. (2014). Social stress and CRF-dopamine interactions in the VTA: Role in long-term escalation of cocaine self-administration. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 34*, 6659-6667. doi:10.1523/JNEUROSCI.3942-13.2014.

Boyson, C., Miguel, T., Quadros, I., DeBold, J. & Miczek, K. (2011). Prevention of social stress-escalated cocaine self-administration by CRF-R1 antagonist in the rat VTA. *Psychopharmacology, 218*, 257-269. doi:10.1007/s00213-011-2266-8.

Brodnik, Z. D., Double, M., España, R. A. & Jaskiw, G. E. (2017). L-tyrosine availability affects basal and stimulated catecholamine indices in prefrontal cortex and striatum of the rat. *Neuropharmacology, 123*, 159-174. doi:10.1016/j.neuropharm.2017.05.030.

Brody, G. H., Yu, T., Chen, Y., Kogan, S. M., Evans, G. W., Windle, M.,... Philibert, R. A. (2013). Supportive family environments, genes that confer sensitivity, and allostatic load among rural African American emerging adults: A prospective analysis. *Journal of Family Psychology, 27*, 22-29. doi:10.1037/a0027829.

Burke, A. & Miczek, K. (2014). Stress in adolescence and drugs of abuse in rodent models: Role of dopamine, CRF, and HPA axis. *Psychopharmacology, 231*, 1557-1580. doi:10.1007/s00213-013-3369-1.

Chaouloff, F. (2013). Social stress models in depression research: What do they tell us? *Cell and Tissue Research, 354*, 179-190. doi:10.1007/s00441-013-1606-x.

Charney, D. S. (2004). Psychobiological mechanisms of resilience and vulnerability: Implications for successful adaptation to extreme stress. *American Journal of Psychiatry, 161*, 195-216. doi:10.1176/appi.ajp.161.2.195.

Chen, R., Kelly, G., Sengupta, A., Heydendael, W., Nicholas, B., Beltrami, S.,... Bhatnagar, S. (2015). MicroRNAs as biomarkers of resilience or vulnerability to stress. *Neuroscience, 305*, 36-48. doi:10.1016/j.neuroscience.2015.07.045.

Chmitorz, A., Kunzler, A., Helmreich, I., Tüscher, O., Kalisch, R., Kubiak, T.,... Lieb, K. (2018). Intervention studies to foster resilience – A systematic review and proposal for a resilience framework in future intervention studies. *Clinical Psychology Review, 59*, 78-100. doi:10.1016/j.cpr.2017.11.002.

Clark, K., Wiley, C. & Bradberry, C. (2013). Psychostimulant abuse and neuroinflammation: Emerging evidence of their interconnection. *Neurotoxicity Research, 23*, 174-188. doi:10.1007/s12640-012-9334-7.

Covington III, H., Kikusui, T., Goodhue, J., Nikulina, E. M., Hammer, R. P. & Miczek, K. A. (2005). Brief social defeat stress: Long lasting effects on cocaine taking during a binge and Zif268 mRNA expression in the amygdala and prefrontal cortex. *Neuropsychopharmacology*, 30, 310-321. doi:10.1038/sj.npp.1300587.

Covington III, H. & Miczek, K. (2001). Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology, 158*, 388-398. doi:10.1007/s002130100858.

Covington III, H. & Miczek, K. (2005). Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: Dissociation from corticosterone activation. *Psychopharmacology, 183*, 331-340. doi:10.1007/s00213-005-0190-5.

Cui, C., Shurtleff, D. & Harris, R. A. (2014). Neuroimmune mechanisms of alcohol and drug addiction. *International Review of Neurobiology, 118,* 1-12. doi:10.1016/B978-0-12-801284-0.00001-4.

Delgado-Palacios, R., Campo, A., Henningsen, K., Verhoye, M., Poot, D., Dijkstra, J.,... Van Der Linden, A. (2011). Magnetic resonance imaging and spectroscopy reveal differential hippocampal changes in anhedonic and resilient subtypes of the chronic mild stress rat model. *Biological Psychiatry, 70,* 449-457. doi:10.1016/j.biopsych.2011.05.014.

Duclot, F., Hollis, F., Darcy, M. J. & Kabbaj, M., (2011). Individual differences in novelty-seeking behavior in rats as a model for psychosocial stress-related mood disorders. *Physiology & Behavior, 104*, 296-305. doi:10.1016/j.physbeh.2010.12.014.

Ferrer-Pérez, C., Castro-Zavala, A., Luján, M. Á., Filarowska, J., Ballestín, R., Miñarro, J.,... Rodríguez-Arias, M. (2019). Oxytocin prevents the increase of cocaine-related responses produced by social defeat. *Neuropharmacology, 146*, 50-64. doi:10.1016/j.neuropharm.2018.11.011.

Ferrer-Pérez, C., Martinez, T., Montagud-Romero, S., Ballestín, R., Reguilón, M. D., Miñarro, J. & Rodríguez-Arias, M. (2018a). Indomethacin blocks the increased conditioned rewarding effects of cocaine induced by repeated social defeat. *PLoS One, 13*, e0209291. doi:10.1371/journal.pone.0209291.

Ferrer-Pérez, C., Reguilón, M. D., Manzanedo, C., Aguilar, M. A., Miñarro, J. & Rodríguez-Arias, M. (2018b). Antagonism of corticotropin-releasing factor CRF 1 receptors blocks the enhanced response to cocaine after social stress. *European Journal of Pharmacology, 823,* 87-95. doi:10.1016/j.ejphar.2018.01.052.

Finnell, J. E., Lombard, C. M., Padi, A. R., Moffitt, C. M., Wilson, L. B., Wood, C. S. & Wood, S. K. (2017). Physical versus psychological social stress in male rats reveals distinct cardiovascular, inflammatory and behavioral consequences. *PLoS One, 12*, e0172868. doi:10.1371/journal.pone.0172868.

Finnell, J. E. & Wood, S. K. (2016). Neuroinflammation at the interface of depression and cardiovascular disease: Evidence from rodent models of social stress. *Neurobiology of Stress, 4*, 1-14. doi:10.1016/j.ynstr.2016.04.001.

García-Pardo, M. P., Rodríguez-Arias, M., Miñarro, J. & Aguilar, M. A. (2016). Effects of social stress on ethanol responsivity in adult mice. *Neuropsychiatry, 6*. doi:10.4172/Neuropsychiatry.1000146.

Gold, P. W., Machado-Vieira, R. & Pavlatou, M. G. (2015). Clinical and biochemical manifestations of depression: Relation to the neurobiology of stress. *Neural Plasticity, 2015*, 581976-11. doi:10.1155/2015/581976.

Golden, S. A., Covington, H. E., Berton, O. & Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nature Protocols, 6*, 1183-1191. doi:10.1038/nprot.2011.361.

Gómez-Lázaro, E., Arregi, A., Beitia, G., Vegas, O., Azpiroz, A. & Garmendia, L. (2011). Individual differences in chronically defeated male mice: Behavioral, endocrine, immune, and neurotrophic changes as markers of vulnerability to the effects of stress. *Stress, 14*, 537-548. doi:10.3109/10253890.2011.562939.

Hawley, D. F., Bardi, M., Everette, A. M., Higgins, T. J., Tu, K. M., Kinsley, C. H. & Lambert, K. G. (2010). Neurobiological constituents of active, passive, and variable coping strategies in rats: Integration of regional brain neuropeptide Y levels and cardiovascular responses. *Stress, 13*, 172-183. doi:10.3109/10253890903144621.

Heffner, T. G., Hartman, J. A. & Seiden, L. S. (1980). A rapid method for the regional dissection of the rat brain. *Pharmacology Biochemistry and Behavior, 13,* 453-456. doi:10.1016/0091-3057(80)90254-3.

Henriques-Alves, A. M. & Queiroz, C. M. (2015). Ethological evaluation of the effects of social defeat stress in mice: Beyond the social interaction ratio. *Frontiers in Behavioral Neuroscience, 9,* 364. doi:10.3389/fnbeh.2015.00364.

Hjemdal, O., Friborg, O. & Stiles, T.C. (2012). Resilience is a good predictor of hopelessness even after accounting for stressful life events, mood and personality (NEO-PI-R). *Scandinavian Journal of Psychology, 53*, 174-180. doi:10.1111/j.1467-9450.2011.00928.x.

Hodes, G. E., Ménard, C. & Russo, S. J. (2016). Integrating interleukin-6 into depression diagnosis and treatment. *Neurobiology of Stress, 4*, 15-22. doi:10.1016/j.ynstr.2016.03.003.

Hollis, F., Duclot, F., Gunjan, A. & Kabbaj, M. (2011). Individual differences in the effect of social defeat on anhedonia and histone acetylation in the rat hippocampus. *Hormones and Behavior, 59*, 331-337. doi:10.1016/j.yhbeh.2010.09.005.

Holly, E. N., Boyson, C. O., Montagud-Romero, S., Stein, D. J., Gobrogge, K. L., DeBold, J. F. & Miczek, K. A. (2016). Episodic social stress-escalated cocaine self-administration: Role of phasic and tonic corticotropin releasing factor in the anterior and posterior ventral tegmental area. *The Journal of Neuroscience, 36,* 4093-4105. doi:10.1523/JNEUROSCI.2232-15.2016.

Koolhaas, J., Bartolomucci, A., Buwalda, B., de Boer, F., Flugge, G., Korte, M. & Fuchs, E. (2011). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology, 31*, 307-321. doi:10.1016/j.yfrne.2010.04.001.

Krishnan, V. (2014). Defeating the fear: New insights into the neurobiology of stress susceptibility. *Experimental Neurology, 261*, 412-416. doi:10.1016/j.expneurol.2014.05.012.

Krishnan, V., Han, M., Graham, A., Graham, D. L., Berton, O., Renthal, W.,... Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell, 131*, 391-404. doi:10.1016/j.cell.2007.09.018.

Kumar, S., Hultman, R., Hughes, D., Michel, N., Katz, B. M. & Dzirasa, K. (2014). Prefrontal cortex reactivity underlies trait vulnerability to chronic social defeat stress. *Nature Communications, 5*, 4537. doi:10.1038/ncomms5537.

Laird, K. T., Krause, B., Funes, C. & Lavretsky, H. (2019). Psychobiological factors of resilience and depression in late life. *Translational Psychiatry, 9*, 88-18. doi:10.1038/s41398-019-0424-7.

Lambert, K. G., Hyer, M. M., Rzucidlo, A. A., Bergeron, T., Landis, T. & Bardi, M. (2014). Contingency-based emotional resilience: Effort-based reward training and flexible coping lead to adaptive responses to uncertainty in male rats. *Frontiers in Behavioral Neuroscience, 8*, 124. doi:10.3389/fnbeh.2014.00124.

Lüthi, A. & Lüscher, C. (2014). Pathological circuit function underlying addiction and anxiety disorders. *Nature Neuroscience, 17*, 1635-1643. doi:10.1038/nn.3849.

Lutter, M., Krishnan, V., Russo, S. J., Jung, S., McClung, C. A. & Nestler, E. J. (2008). Orexin signaling mediates the antidepressant-like effect of calorie restriction. *Journal of Neuroscience, 28*, 3071-3075. doi:10.1523/JNEUROSCI.5584-07.2008.

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G.,... Maj, M. (2009). The inflammatory &

neurodegenerative (I&ND) hypothesis of depression: Leads for future research and new drug developments in depression. *Metabolic Brain Disease, 24*, 27-53. doi:10.1007/s11011-008-9118-1.

Maldonado, C., Rodríguez-Arias, M., Castillo, A., Aguilar, M. & Miñarro, J. (2006). Gamma-hydroxybutyric acid affects the acquisition and reinstatement of cocaine-induced conditioned place preference in mice. *Behavioural Pharmacology, 17*, 119-131. doi:10.1097/01.fbp.0000190685.84984.ec.

Manzanedo, C., Aguilar, M. A., Rodríguez-Arias, M. & Miñarro, J. (2001). Effects of dopamine antagonists with different receptor blockade profiles on morphine-induced place preference in male mice. *Behavioural Brain Research, 121*, 189-197. doi:10.1016/S0166-4328(01)00164-4.

Martínez, M., Miñarro, J. & Simón, V. M. (1991). Análisis etoexperimental de la conducta agonística en ratones. *Psicológica, 12*, 1-22.

McGloin, J. & Widom, C.S. (2001). Resilience among abused and neglected children grown up. *Development and Psychopathology, 13*, 1021-1038. doi:10.1017/S095457940100414X.

Menard, C., Pfau, M. L., Hodes, G. E. & Russo, S. J. (2017). Immune and neuroendocrine mechanisms of stress vulnerability and resilience. *Neuropsychopharmacology, 42*, 62-80. doi:10.1038/npp.2016.90.

Miczek, K. A., Yap, J. J. & Covington, H. E. (2008). Social stress, therapeutics and drug abuse: Preclinical models of escalated and depressed intake. *Pharmacology and Therapeutics, 120*, 102-128. doi:10.1016/j.pharmthera.2008.07.006.

Montagud-Romero, S., Daza-Losada, M., Vidal-Infer, A., Maldonado, C., Aguilar, M. A. & Miñarro, J. (2014). The novelty-seeking phenotype modulates the long-lasting effects of intermittent ethanol administration during adolescence. *PLoS One, 9*, e92576. doi:10.1371/journal.pone.0092576.

Montagud-Romero, S., Reguilon, M. D., Roger-Sanchez, C., Pascual, M., Aguilar, M. A., Guerri, C.,... Rodríguez-Arias, M. (2016a). Role of dopamine neurotransmission in the long-term effects of repeated social defeat on the conditioned rewarding effects of cocaine. *Progress in Neuropsychopharmacology & Biological Psychiatry, 71*, 144-154. doi:10.1016/j.pnpbp.2016.07.008.

Montagud-Romero, S., Montesinos, J., Pascual, M., Aguilar, M. A., Roger-Sanchez, C., Guerri, C.,... Rodríguez-Arias, M. (2016b). Up-regulation of histone acetylation induced by social defeat mediates the conditioned rewarding effects of cocaine. *Progress in Neuropsychopharmacology & Biological Psychiatry, 70*, 39-48. doi:10.1016/j.pnpbp.2016.04.016.

Moreira, F. P., Medeiros, J. R. C., Lhullier, A. C., de Mattos Souza, L. D., Jansen, K., Portela, L. V.,... Oses, J. P. (2016). Cocaine abuse and effects in the serum levels of cytokines IL-6 and IL-10. *Drug and Alcohol Dependence, 158*, 181-185. doi:10.1016/j.drugalcdep.2015.11.024.

Newman, E. L., Leonard, M. Z., Arena, D. T., de Almeida, R. M. M. & Miczek, K. A. (2018). Social defeat stress and escalation of cocaine and alcohol consumption: Focus on CRF. *Neurobiology of Stress, 9*, 151-165. doi:10.1016/j.ynstr.2018.09.007.

Pearson-Leary, J., Eacret, D., Chen, R., Takano, H., Nicholas, B. & Bhatnagar, S. (2017). Inflammation and vascular remodeling in the ventral hippocampus contributes to vulnerability to stress. *Translational Psychiatry, 7*, e1160. doi:10.1038/tp.2017.122.

Pérez-Tejada, J., Arregi, A., Gómez-Lázaro, E., Vegas, O., Azpiroz, A. & Garmendia, L. (2013). Coping with chronic social stress in mice: Hypothalamic-pituitary-adrenal/sympathetic-adrenal-medullary axis activity, behavioral changes and effects of antalarmin treatment: Implications for the study of stress-related psychopathologies. *Neuroendocrinology, 98*, 73-88. doi:10.1159/000353620.

Pfau, M. L., & Russo, S. J. (2015). Peripheral and central mechanisms of stress resilience. *Neurobiology of Stress, 1*, 66-79. doi:10.1016/j.ynstr.2014.09.004.

Pfau, M. L. & Russo, S. J. (2016). Neuroinflammation regulates cognitive impairment in socially defeated mice. *Trends in Neurosciences,* 39, 353-355. doi:10.1016/j.tins.2016.04.004.

Polter, A. M. & Kauer, J. A. (2014). Stress and VTA synapses: Implications for addiction and depression. *European Journal of Neuroscience, 39*, 1179-1188. doi:10.1111/ejn.12490.

Réus, G. Z., Fries, G. R., Stertz, L., Badawy, M., Passos, I. C., Barichello, T.,... Quevedo, J. (2015). The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience, 300,* 141-154. doi:10.1016/j.neuroscience.2015.05.018.

Rodríguez-Arias, M., Montagud-Romero, S., Carrión, A. M. G., Ferrer-Pérez, C., Pérez-Villalba, A., Marco, E.,... Miñarro, J. (2018). Social stress during adolescence activates long-term microglia inflammation insult in reward processing nuclei. *PLoS One, 13*, e0206421. doi:10.1371/journal.pone.0206421.

Rodríguez-Arias, M., Montagud-Romero, S., Rubio-Araiz, A., Aguilar, M. A., Martín-García, E., Cabrera, R.,... Miñarro, J. (2017). Effects of repeated social defeat on adolescent mice on cocaine-induced CPP and self-administration in adulthood: Integrity of the blood–brain barrier. *Addiction Biology, 22*, 129-141. doi:10.1111/adb.12301.

Rodríguez-Arias, M., Navarrete, F., Blanco-Gandia, M. C., Arenas, M. C., Bartoll-Andrés, A., Aguilar, M. A.,... Manzanares, J. (2016). Social defeat in adolescent mice increases vulnerability to alcohol consumption. *Addiction Biology, 21*, 87-97. doi:10.1111/adb.12184.

Rodríguez-Arias, M., Vaccaro, S., Arenas, M. C., Aguilar, M. A. & Miñarro, J. (2015). The novelty-seeking phenotype modulates the long-lasting effects of adolescent MDMA exposure. *Physiology & Behavior, 141*, 190-198. doi:10.1016/j.physbeh.2015.01.023.

Russo, S. J., Murrough, J. W., Han, M., Charney, D. S. & Nestler, E. J. (2012). Neurobiology of resilience. *Nature Neuroscience, 15*, 1475-1484. doi:10.1038/nn.3234.

Tornatzky, W. & Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology & Behavior, 53*, 983-993. doi:10.1016/0031-9384(93)90278-N.

Tzschentke, T. M. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. *Addiction Biology, 12*, 227-462. doi:10.1111/j.1369-1600.2007.00070.x.

Vidal-Infer, A., Aguilar, M. A., Miñarro, J. & Rodríguez-Arias, M. (2012). Effect of intermittent exposure to ethanol and MDMA during adolescence on learning and memory in adult mice. *Behavioral and Brain Functions, 8*, 32. doi:10.1186/1744-9081-8-32.

Wang, J., Hodes, G. E., Zhang, H., Zhang, S., Zhao, W., Golden, S. A.,... Pasinetti, G. M. (2018). Epigenetic modulation of inflammation and synaptic plasticity promotes resilience against stress in mice. *Nature Communications, 9*, 477. doi:10.1038/s41467-017-02794-5.

Wood, S. K. & Bhatnagar, S. (2015). Resilience to the effects of social stress: Evidence from clinical and preclinical studies on the role of coping strategies. *Neurobiology of Stress, 1*, 164-173. doi:10.1016/j.ynstr.2014.11.002.

Wood, S. K., Walker, H. E., Valentino, R. J. & Bhatnagar, S. (2010). Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: Role of corticotropin-releasing factor. *Endocrinology, 151*, 1795-1805. doi:10.1210/en.2009-1026.

Wood, S. K., Wood, C. S., Lombard, C. M., Lee, C. S., Zhang, X., Finnell, J. E. & Valentino, R. J. (2015). Inflammatory factors mediate vulnerability to a social stress-induced depressive-like phenotype in passive coping rats. *Biological Psychiatry, 78*, 38-48. doi:10.1016/j.biopsych.2014.10.026.

Yap, J., Chartoff, E., Holly, E., Potter, D., Carlezon Jr, W. & Miczek, K. (2015). Social defeat stress-induced sensitization and escalated cocaine self-administration: The role of ERK signaling in the rat ventral tegmental area. *Psychopharmacology, 232*, 1555-1569. doi:10.1007/s00213-014-3796-7.

Zhan, G., Huang, N., Li, S., Hua, D., Zhang, J., Fang, X., ... Yang, C. (2018). PGC-1α–FNDC5–BDNF signaling pathway in skeletal muscle confers resilience to stress in mice subjected to chronic social defeat. *Psychopharmacology, 235*, 3351-3358. doi:10.1007/s00213-018-5041-2.