

The negative allosteric modulator of mGluR5, MPEP, potentiates the rewarding properties of cocaine in priming-induced reinstatement of CPP

El modulador alostérico negativo de los mGluR5, MPEP, potencia la reinstauración de la preferencia condicionada inducida con priming de cocaína

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Abstract

Cocaine addiction is a chronic disorder with high relapse rates; therefore, understanding the neuronal mechanisms underlying drug-seeking during relapse is a priority to develop targeted pharmacotherapy. The metabotropic glutamate receptor 5 (mGluR5) seems to be involved in the reinstatement induced by cocaine-associated cues. The main objective of the study was to evaluate the efficacy of MPEP, a negative allosteric modulator of mGluR5, in attenuating or potentiating the reinstatement induced by priming doses of cocaine in the CPP paradigm, ultimately to further knowledge regarding the role of the mGluR5 in relapse into cocaine abuse. OF1 mice (48 female and 48 male) were conditioned in the CPP paradigm with cocaine (20 mg/kg) and were exposed to an extinction program. We evaluated the efficacy of MPEP (30 mg/kg) in blocking the successive cocaine-priming reinstatements in the CPP when extinction of the conditioning preference was confirmed. MPEP did not block the reinstatement of priming cocaine-induced CPP, but increased the potential of cocaine for reinstating conditioning preference. The contingent administration of MPEP with cocaine increased the drug-seeking behaviour and the number of reinstatements with priming doses of cocaine. Moreover, MPEP produced cross reinstatement of cocaine-induced CPP. Rather than preventing the reinstatements of conditioned preference induced by priming doses of cocaine, MPEP increased them. These findings may help to understand the role of mGluR5 in the relapse into cocaine abuse.

Key Words: MPEP; Cocaine; CPP; Reinstatement; mGluR5.

Resumen

La adicción a la cocaína es un trastorno crónico con un alto índice de recaídas; por tanto, es prioritario entender los mecanismos neurales implicados en la búsqueda de la droga durante la recaída para desarrollar farmacoterapias eficaces. El receptor metabotrópico 5 del glutamato (mGluR5) parece estar implicado en la reinstauración inducida por las claves asociadas a la cocaína. El objetivo principal de este estudio fue profundizar en el papel del receptor mGluR5 en la recaída en el consumo de cocaína, evaluando el efecto del MPEP, un modulador alostérico negativo del mGluR5, sobre la reinstauración inducida por un *priming* de cocaína en el paradigma del condicionamiento de la preferencia de lugar (CPL). Ratones OF1 (48 machos y 48 hembras) fueron condicionados en el paradigma del CPL con cocaína (20 mg/kg) y expuestos a un programa de extinción. Cuando la extinción de la preferencia condicionada fue confirmada, se evaluó la eficacia del MPEP (30 mg/kg) para bloquear las sucesivas reinstauraciones mediante *priming* de cocaína en el CPL. La administración contingente de MPEP con la cocaína en el CPL incrementó la conducta de búsqueda de la droga y el número de reinstauraciones. Además, la administración solo de MPEP produjo reinstauración cruzada en el CPL inducido por cocaína. Por tanto, el MPEP no solo no previno, sino que incrementó las reinstauraciones de la preferencia condicionada inducida por *priming* de cocaína. Estos resultados pueden ayudar a entender el papel del mGluR5 en la recaída al consumo de cocaína.

Palabras clave: MPEP; Cocaína; CPL; Reinstauración; mGluR5.

Received: March 2018; Accepted: December 2018.

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Cocaine addiction is a chronic disorder with high relapse rates (EMCDDA, 2016), and, currently, there is no approved treatment for its addiction. Understanding the neuronal mechanisms that underlie drug-seeking during relapse is a priority in order to develop targeted pharmacotherapy for its prevention (Georgiou et al., 2015; McReynolds, Peña, Blacktop & Mantsch, 2014).

Preclinical and clinical research suggests that glutamatergic transmission plays a pivotal role in drug-seeking and relapse into abuse (see reviews Clewa & Olive, 2012; Olive, 2009; Pomierny-Chamiólo et al., 2014). In particular, the metabotropic glutamate receptor 5 (mGluR5) is involved in synaptic plasticity (Brown et al., 2012; Schmidt & Pierce, 2010), and it has been suggested that it is necessary for drug-seeking behaviour during relapse into cocaine (Backstrom & Hyytia, 2006, 2007; Brown et al., 2012; Keck et al., 2014; Kumaresan et al., 2009; Schmidt, Kimmey, Arreola & Pierce, 2015). Recently, numerous alterations in mGlu5 receptors' density and affinity in rat brain structures, such as the dorsal striatum and the nucleus accumbens (NAcc) shell, have been observed after the administration of cocaine, changes related to either the pharmacological or motivational properties of drug (Pomierny-Chamiólo, Miszkiewicz, Frankowska, Bystrowska & Filip, 2017). Specifically, MPEP (2-methyl-6-(phenylethynyl)-pyridine), a negative allosteric modulator of mGluR5 (Olive, 2009), is an active ligand via systemic administration with a potent selective mGluR5 noncompetitive antagonist (Gasparini et al., 1999; Pomierny-Chamiólo et al., 2014), and it has been proven effective in reducing drug intake, reward, and relapse into cocaine (Li et al., 2018). A recent systematic review has come to the conclusion that MPEP reduces self-administration of cocaine, indicating its therapeutic potential in the treatment of addictive disorders (Mihov & Hasler, 2016). The systemic injection of MPEP attenuated both priming-induced and cue-induced reinstatements of cocaine (Backstrom & Hyytia, 2006; Kumaresan et al., 2009), nicotine (Bespalov et al., 2005) and alcohol (Backstrom, Bachteler, Koch, Hyytia & Spanagel, 2004) in rodents with the self-administration model. The mechanisms behind this decrease in the self-administration of drugs are not clear. Treatment with MPEP increased the potency of drugs to induce conditioned place preference (CPP) in rats, such as ketamine, heroin, nicotine and cocaine (Rutten, Van der Kam, De Vry, Bruckmann & Tzschentke, 2011; Van der Kam, De Vry & Tzschentke, 2009a). Because of this, it has been suggested that MPEP reduces the self-administration of drugs, since it increases their rewarding effects (Van der Kam et al., 2009a; Van der Kam, De Vry & Tzschentke, 2009b). Specifically, MPEP's effect on the acquisition of CPP induced by several doses of cocaine has been assayed; finding that the administration of MPEP before the drug reduced the lowest necessary dose of cocaine to induce a

statistically significant CPP to half its value (Rutten et al., 2011). However, to date, the effects of MPEP on the cocaine priming-induced reinstatement of CPP have not been tested. Two main versions of the reinstatement model are employed to study relapse into drugs; one based on the operant self-administration and the other on the classical CPP, in which preference for a drug-paired environment can also be extinguished and subsequently reinstated by drug priming injections (Manzanedo, Aguilar, Rodríguez-Arias & Miñarro 2001a). Although CPP evaluates drug reinforcement differently, its results provide supplementary information to that offered by the self-administration paradigm (Aguilar, Rodríguez-Arias & Miñarro, 2009; Sanchis-Segura & Spanagel, 2006; Tzschentke, 2007).

For these reasons, the aim of the present study was to evaluate the efficacy of MPEP in attenuating or potentiating the reinstatement induced by priming doses of cocaine in the CPP paradigm, ultimately to further knowledge regarding the role of the mGluR5 in the relapse into cocaine abuse.

Material and Methods

Subjects

A total of 48 female and 48 male mice of the OF1 strain (Charles River, France) were employed. The animals arrived at the laboratory at postnatal day (PND) 42, and were all housed in groups of four in plastic cages (28 cm length × 28 cm width × 14.5 cm height) under the following conditions: constant temperature (21±2 °C), a relative humidity of 60%, a 12h non-inverted light cycle (lights on from 8:00 to 20:00), and food and water available *ad libitum* (except during behavioural tests). Procedures involving mice and their care were conducted in conformity with national, regional and local laws and regulations, which are in accordance with Directive 2010/63/EU of the European Parliament and the council of September 22, 2010 on the protection of animals used for scientific purposes. The Animal Use and Care Committee of the University of Valencia approved the present study (2015/VSC/PEA/00103).

Drugs

Cocaine hydrochloride (Laboratorios Alcaliber S. A. Madrid, Spain) at doses of 20 mg/kg (acquisition of CPP) and at doses of 10, 5, 2.5, 1.25 or 0.625 mg/kg (reinstatement test), and MPEP (Research Biochemicals International, Natick, USA) at doses of 30, 15, 7.5, 3.25 or 1.625 mg/kg (reinstatement test) were diluted both in physiological saline (0.9% NaCl) and administered i.p. in a volume of 0.01 ml/g (Manzanedo, Aguilar, Miñarro & Rodríguez-Arias, 2011). Control groups were injected with the physiological saline.

Apparatus

The eight identical Plexiglas boxes employed for the conditioned place preference (CPP) test have two equally

sized compartments (30.7 cm length × 631.5 cm width × 634.5 cm height) separated by a grey central area (13.8 cm length × 631.5 cm width × 634.5 cm height). The compartments have different coloured walls (black vs white) and distinct floor textures (fine grid in the black compartment and wide grid in the white one). Four infrared light beams in each compartment of the box and six in the central area allowed the recording of the position of the animal and its crossings from one compartment to the other. The equipment was controlled by two IBM PC computers using MONPRE 2Z software (CIBERTEC, SA, Spain).

Procedures and experimental design

The reinstatement of drug-conditioned place preference described in Aguilar et al. (2009) was the animal model used to measure the relapse. The CPP version of the reinstatement model consists of three phases, the acquisition, the extinction and the reinstatement of the preference by drug priming (Blanco-Gandía, Aguilar, Miñarro & Rodríguez-Arias, 2018).

Acquisition of CPP: The procedure, unbiased in terms of initial spontaneous preference, was performed as described previously (Manzanedo, Aguilar, Rodríguez-Arias & Miñarro, 2001b). To summarize, after an acclimatization period of at least 5 days (from PND 47), animals were handled briefly on each of the 3 days preceding initiation of the CPP, which consisted of three phases. In the first phase or Pre-Conditioning (Pre-C), the animals were allowed access to both compartments of the apparatus for 15 minutes (900 seconds) per day on 3 consecutive days. On day 3, the time spent by an animal in each compartment during a period of 900 seconds was recorded. A total of 4 male mice showed a strong unconditioned aversion (less than 33% of the session time) or preference (more than 67%) for one of the compartments and, therefore, they were excluded from the study. In each group, half the animals received the drug or vehicle in one compartment and the other half in the other compartment. Four groups were established for conditioning: two with cocaine (Coc-female n=37 and Coc-male n=33) and two with physiological saline (control groups: Sal-female n=11 and Sal-male n=11). After assigning the compartments, an analysis of variance revealed no significant difference between the time spent in the drug-paired vs. non-paired compartments during the Pre-C phase [$F(1,90)=2.103$; $p<0.150$]. In the second phase (Conditioning), animals were conditioned with cocaine or physiological saline through four pairings with the respective compartment, which underwent two pairings per day. The animals received an injection of 20 mg/kg cocaine (experimental group) or physiological saline (control group) and, immediately afterwards, were confined to the drug-paired compartment for 30 min. Later, after an interval of 4h, they received an injection of physiological saline immediately before confinement to the

vehicle-paired compartment for 30 min. The central area was made inaccessible during conditioning by lowering the guillotine doors. During the third phase or post-conditioning (Post-C), the guillotine doors separating the two compartments were removed and the time spent by the untreated mice in each compartment during a period of 900 seconds was recorded. The difference in seconds between the time spent in the drug-paired compartment in the Post-C test and the time spent in the same compartment in the Pre-C test is considered to be a measure of the degree of conditioning induced by the drug. If this difference is positive, then the drug has induced a preference for the drug-paired compartment, whereas the opposite indicates the development of an aversion.

Extinction of CPP (Extinc): After the Post-C test, twice a week, the mice underwent an extinction session, which consisted of placing the animals in the apparatus (without guillotine doors separating the compartments) for a period of 15 min until the time spent in the drug-paired compartment by each group of animals was similar to that recorded in the Pre-C session. The extinction of CPP was always confirmed in a subsequent session performed 24h after the last extinction session. Thus, all the animals in each group received the same number of extinction sessions, independently of their individual scores, since the extinction criterion was a lack of significant differences with respect to Pre-C values from the group. Control groups were subjected to the same protocol extinction and number sessions. The extinction of conditioning was considered to have occurred when there was no significant difference in the time spent on the site associated with the administration of cocaine in comparison to the Pre-C, but there were differences with the Post-C.

Reinstatement of CPP (Reinst): The effects of a priming dose of cocaine, MPEP, or cocaine+MPEP were evaluated 24 hours after extinction had been confirmed. Reinstatement tests were the same as those carried out in Post-C (free ambulation for 15 minutes), except that animals were tested 30 minutes after administration of MPEP and/or 15 minutes after administration of cocaine according to the respective treatment. The reinstatement of preference induced by cocaine was considered to occur when the time that each animal spent in the cocaine-paired compartment was significantly higher in the Reinst test in comparison to the Pre-C and Extinc phases. When reinstatement of the preference was achieved, after a subsequent extinction process, a new reinstatement test was conducted with progressively lower doses of the drug (half the dose used in the previous priming).

When extinction of the groups conditioned with cocaine was confirmed after 21 sessions for the females or 19 sessions for the male, mice were grouped into six groups (three female and three male) according to the treatment they will receive in the reinstatement-test of conditioned

preference. Besides, the control groups (conditioned with saline) received cocaine 10 mg/kg in the reinstatement-test. Therefore, the following experimental groups were established: Sal-Coc (female n=11 and male n=11), Coc-Coc (female n=12 and male n=11), Coc-MPEP (n=12 female and n=11 male), and, Coc-Coc+MPEP (female n=13 and male n=11). The doses of drug used for priming were cocaine 10 and/or MPEP 30 (Reinst 1); cocaine 5 and/or MPEP 15 (Reinst 2); cocaine 2.5 and/or MPEP 7.5 (Reinst 3); cocaine 1.25 and/or MPEP 3.25 (Reinst 4); cocaine 0.625 and/or MPEP 1.625 (Reinst 5).

Statistical analyses

The time spent in the drug-paired compartment during pre- and post-conditioning was analysed by means of a mixed analysis of variance (ANOVA) with two between variables –Sex, with two levels (female or male) and Conditioning, with two levels (physiological saline or cocaine 20 mg/kg)– and one within variable –Days, with two levels (Pre-C, Post-C). To evaluate the effect of the treatment that the animals received on the reinstatement test, an ANOVA was performed with two between variables –Sex, with two levels (female or male) and Treatment, with three levels (cocaine 10 mg/kg, cocaine 10 mg/kg plus MPEP 30 mg/kg, and MPEP 30 mg/kg)– and a within variable –Days, with four levels (Pre-C, Post-C, Extinct1 and Reinst1)–. The paired samples “t” Student test was used to determine the extinction of conditioned preference in each group as well as the reinstatement by a drug priming dose. A Bonferroni test was used for post hoc comparisons of ANOVAs and all analyses were conducted using the Statistical Package for the Social Sciences (SPSS) 20.0 for Windows (Systat Software Inc., Chicago, IL, USA).

Results

The ANOVA to evaluate the sex differences in the rewarding conditioned effect of cocaine revealed a main effect of Days [$F(1.88)=31.395$; $p<0.0001$], an effect of the interaction Days*Conditioning [$F(1.88)=31.009$; $p<0.0001$] and a significant effect of the interaction Days*Conditioning*Sex [$F(1.88)=3.792$; $p<0.05$]. The animals (both female and male) that had received cocaine showed a conditioned place preference ($p<0.0001$), which was not observed in those that had received physiological saline. Moreover, the male mice stayed longer in the compartment associated with cocaine compared to the females ($p<0.022$).

The ANOVA to evaluate the treatment's effect on the Reinstatement test showed a significant effect of the variable Days [$F(1.79)=4.742$; $p<0.03$] and the interaction Days*Treatment [$F(3.79)=3.668$; $p<0.01$], merging males and females together in the same groups (see Figure 1), as no significant differences in the variable Sex or in their interactions were observed. The distribution of groups for

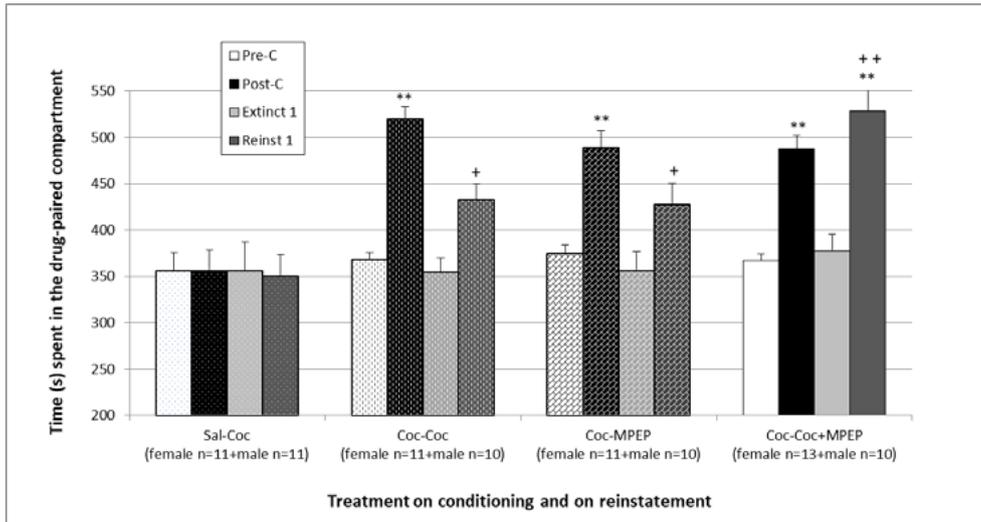
treatment on Reinst-1 was homogeneous, since no significant differences among groups conditioned with cocaine 20mg/kg were found in the Pre-C, Post-C and Extinct-1 tests. As expected, the groups conditioned with saline (control groups) did not show a conditioned preference for any compartment after the administration of cocaine 10 mg/kg in the Reinst-1 test. However, cocaine priming in the Reinst-1 test significantly increased the time spent by the animals in the drug paired compartment in the Coc-Coc group in comparison with the Extinct-1 test ($p<0.01$), and in the group treated with MPEP 30 mg/kg in comparison with the Extinct-1 and the Pre-C tests (Coc-Coc+MPEP, $ps<0.0001$). Moreover, the sole administration of MPEP reinstated the CPP too, since it increased the time spent by the animals in the drug paired compartment in comparison with the Extinct-1 test (Coc-MPEP, $p<0.01$) (see Figure 1).

The results of successive extinctions and reinstatement tests in the groups conditioned with cocaine are shown in the Figure 2 separated by sex, since female and male presented differences in the reinstatements. Once the preference was extinguished after Reinst-1 in each group (Extinct-2 test), a priming of cocaine 5mg/kg reinstated CPP in Coc-Coc males [Extinct-2 vs. Reinst-2: $t(8)=-2.561$; $p<0.034$], but not in females. Males of this group did not achieve reinstatement of the preference again with a dose of 2.5mg/kg. After their corresponding extinction, the females of the Coc-MPEP group showed preference with just one priming of MPEP 15mg/kg [Extinct-2 vs. Reinst-2: $t(11)=-3.363$; $p<0.006$], which was not the case with male mice. The administration of MPEP 7.5mg/kg did not reinstate the preference in females again (Reinst-3). After their corresponding extinctions, the Coc-Coc+MPEP groups showed preference with a priming of cocaine 5mg/kg plus MPEP 15mg/kg [Extinct-2 vs. Reinst-2: female $t(11)=-3.965$; $p<0.002$; and male $t(8)=-2.460$; $p<0.039$], with a priming of cocaine 2.5mg/kg plus MPEP 7.5mg/kg [Extinct-3 vs. Reinst-3: female $t(11)=0.851$; $p<0.001$; and male $t(8)=-3.004$; $p<0.017$], and with a priming of cocaine 1.25mg/kg plus MPEP 3.25mg/kg only in male mice [Extinct-4 vs. Reinst-4: $t(8)=-3.177$; $p<0.013$]. The administration of cocaine 0.625mg/kg plus MPEP 1.875mg/kg did not reinstate the preference in males again (Reinst-5).

The number of sessions required to reach extinction of the conditioned preference before each reinstatement was, in the case of the females: Coc-Coc group, 7 sessions; Coc-MPEP group, 2 and 5 sessions; Coc-Coc+MPEP group, 2, 2, and 2 sessions; and in the case of the males: Coc-Coc group, 4 and 3 sessions; Coc-MPEP group, 10 sessions; Coc-Coc+MPEP group, 3, 3, 5 and 2 sessions.

Discussion

The effects of MPEP, a negative allosteric modulator of mGluR5, on the reinstatement of cocaine-induced CPP in



Note. ** $p < 0.001$ vs. Pre-C. + $p < 0.05$ ++ $p < 0.001$ vs. Extinct 1.

Figure 1. Effect of MPEP on the reinstatement of cocaine-induced CPP. Bars represent the mean (\pm SEM) time spent in the drug-paired compartment before conditioning sessions (white), after conditioning sessions (black), when there was extinction (stippled) and after priming of cocaine, MPEP or cocaine plus MPEP (gray).

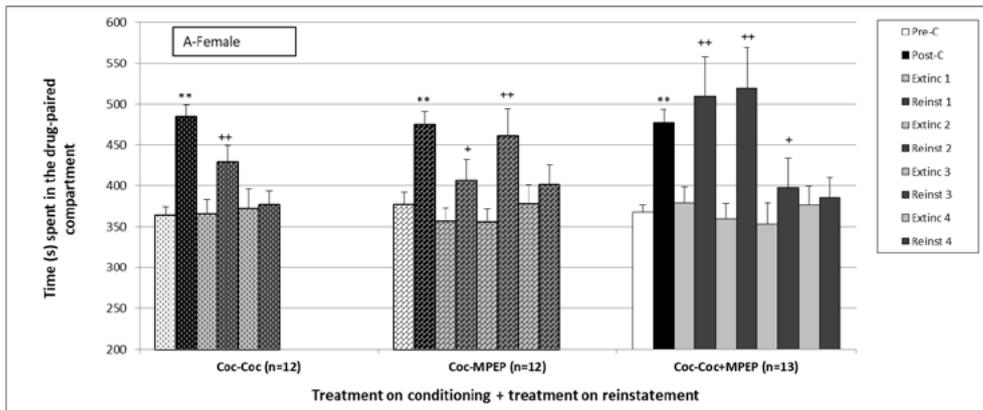


Figure 2A.

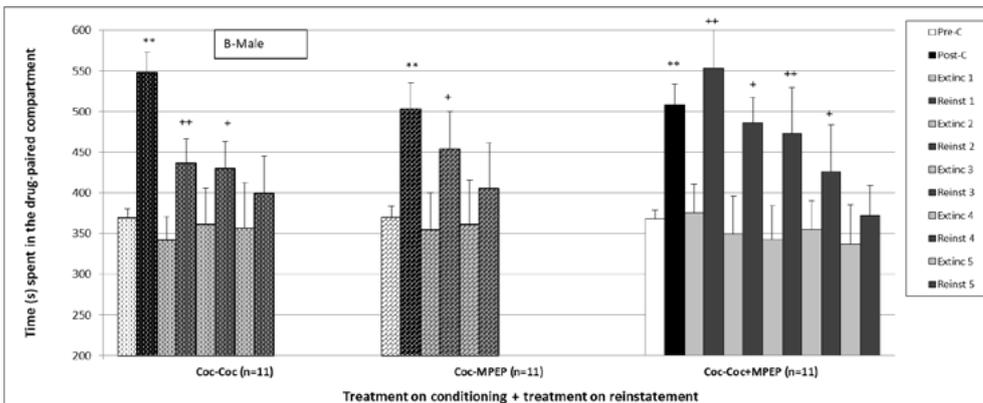


Figure 2B.

Note. ** $p < 0.001$ vs. Pre-C. + $p < 0.05$ and ++ $p < 0.01$ vs. previous Extinc.

Doses in mg/kg in each reinstatement: Reinst 1: cocaine 10 and/or MPEP 30; Reinst 2: cocaine 5 and/or MPEP 15; Reinst 3: cocaine 2.5 and/or MPEP 7.5; Reinst 4: cocaine 1.25 and/or MPEP 3.75; Reinst 5: cocaine 0.625 and/or MPEP 1.875

Figure 2. Effects the priming of cocaine, cocaine plus MPEP or MPEP alone in the reinstatement of CPP induced by cocaine on female (A) and male mice (B). Bars represent the mean (\pm SEM) time spent in the drug-paired compartment before conditioning sessions (white), after conditioning sessions (black), when there was extinction (stippled) and each reinstatement (gray).

female and male mice have been evaluated for the first time to our knowledge. Our results show that MPEP did not block the reinstatement of CPP induced by cocaine priming, but it increased the cocaine potential for reinstating conditioning preference. The administration of MPEP alongside a priming dose of cocaine produced a higher number of reinstatements than the administration of a single cocaine priming dose, since MPEP caused ineffective doses of cocaine to induce the reinstatement of the conditioned preference. Moreover, MPEP also produced cross reinstatement of cocaine-induced CPP both in females and males.

In this work, the administration of a priming dose of cocaine 10 mg/kg reinstated, as expected, the conditioning preference induced by cocaine in accordance with previous studies (Bracci et al., 2013; Rodríguez-Arias, Castillo, Daza-Losada, Aguilar & Miñarro, 2009; Titomanlio et al., 2013). However, not only did MPEP not inhibit the reinstatement of CPP induced by a priming dose of cocaine, but it caused reinstatement in the drug-seeking behaviour with much lower priming doses of cocaine (2.5 mg/kg), which do not produce reinstatement of conditioned preference when cocaine is administered alone. Therefore, the administration of MPEP before cocaine has shown an augmentation in the effects of a cocaine priming dose. These results are in agreement with those observed in other studies (Rutten et al. 2011; Van der Kam et al. 2009a; 2009b) that proposed that MPEP did not block the reinforcing effects of the tested drugs in the CPP paradigm, but that it rather potentiated its rewarding properties. MPEP potentiated the acquisition of CPP induced by addictive drugs with different mechanisms of action, such as ketamine, heroin (Van der Kam et al., 2009a), nicotine and cocaine (Rutten et al., 2011); it delayed the extinction of ketamine-induced CPP and reinstated CPP induced by both ketamine and heroin in rats (Van der Kam et al., 2009a).

Moreover, the results of the present study have shown that MPEP by itself induces reinstatement of extinguished preference in cocaine-induced CPP, at doses of 30, 15 and 7.5 mg/kg, which is indicating of a cross-reinstatement. This effect of MPEP on the conditioned preference reinstatement was also observed in CPP induced by both ketamine and heroin in rats (Van der Kam et al., 2009a). Previous studies reported a lack of rewarding effect of 30 mg/kg MPEP in mice, since the i.p. administration of MPEP did not induce a CPP development by itself (Herzig, Capuani, Kovar & Schmidt, 2005; Popik & Wrobel, 2002). However, our results reveal a cross-reinstatement with this dose of MPEP after conditioning with cocaine, even with lower doses. Similarly, another study showed that the i.v. administration of MPEP (3 and 10 mg/kg) induced conditioned place preference (Van der Kam et al., 2009b), which indicates that MPEP presents rewarding properties by itself, as confirmed by the results of the present study. The administration of MPEP has been found to raise DA levels in the medial prefrontal cortex and

NAcc (Chau, Soderpalm & Ericson, 2011; Homayoun, Stefani, Adams, Tamagan & Moghaddam, 2004). MPEP could stimulate the DA system by activating the glycine receptors (Chau et al., 2011) or by inhibiting the activity of the mono-amino-oxidase A (MAO-A) (Schmidt & Pierce, 2010). These actions on the DA system could explain why MPEP increases the effects of cocaine as well as producing cross-reinstatement, as observed in this study.

Additionally, the results of the present study have shown sex differences in the CPP induced by cocaine. Male mice showed a conditioned preference induced by cocaine (20 mg/kg) higher than that in females, since they spent significantly more time in the drug-paired compartment on the Post-C test. This sex difference in the CPP paradigm, which has not been observed in previous studies (Bobzean, Dennis, Addison & Perrotti, 2010; Hilderbrand & Lasek, 2014; Mateos-García et al., 2015) may be due to the elevated number of subjects employed by group ($n > 30$). However, this higher preference in males is also observed in the higher number of reinstatement in conditioned preference with lower doses of cocaine. These results seem to be in accordance with other results showing that males take longer to extinguish cocaine-induced CPP than their female counterparts (Hilderbrand & Lasek, 2014). The study of sex differences is an emergent field (Bobzean, Dennis & Perrotti, 2014), and these differences have been observed at every phase of the addiction process. They are robust and reliable, being observed in several species, such as rodents and humans, but they are dependent on many factors (Carroll & Lynch, 2016). Generally speaking, sex differences are more likely to be observed under certain conditions, such as low doses of drugs or, as in this case, with an elevated number of evaluated subjects (Hilderbrand & Lasek, 2014).

A potential therapeutic action of mGluR5 negative allosteric modulators, such as MPEP, has been suggested (Mihov & Hasler, 2016), since this drug reduces the self-administration of cocaine at doses which do not impair food self-administration. Specifically, MPEP significantly attenuated the reinstatement of cocaine-seeking in the self-administration paradigm when it was both induced by cues associated with the drug (Backstrom & Hyttia, 2006; Li et al., 2018) and a priming injection of cocaine (Kumaresan et al., 2009; Lee, Platt, Rowlett, Adewale & Spealman, 2005; Li et al., 2018), without affecting natural reinforcements, such as food or sucrose (Herzig et al., 2005). These results seem to stem from its action on NAcc, since administration of MPEP directly into the NAcc attenuated cocaine priming-induced reinstatement of drug-seeking (Schmidt et al., 2015). The role of the glutamatergic system in cocaine priming-induced reinstatement of drug-seeking behaviour is known. The stimulation of glutamatergic pyramidal neurons from the medial prefrontal cortex to the NAcc appears to promote

cocaine priming-induced reinstatement of drug seeking (Schmidt & Pierce, 2010). Specifically, mGlu5 receptors, present in the NAcc, have been implicated in the reinforcing effects of cocaine (Pomierny-Chamiólo et al., 2014; 2017; Li et al., 2018). Recently, it has been shown that the reduction of cocaine self-administration induced by MPEP is associated with an elevation of extracellular glutamate in the NAcc via a retrograde eCB-CB1 receptor mechanism. Therefore, it is speculated that a presynaptic glutamate/CB1 mechanism may be the cause of the anti-cocaine therapeutic effects of mGluR5 antagonists in animal models of drug relapse (Li et al., 2018). However, using MPEP as a treatment for addiction does not seem advisable because of its undesirable off-target effects, possibly due to its actions as an antagonist of NMDA receptors and an inhibitor of monoamine oxidase A (Schmidt & Pierce, 2010). Additionally, studies using the CPP paradigm seem to demonstrate MPEP's lack of efficacy in decreasing the drug-seeking behaviour, while also increasing the rewarding effects of drugs, as the results of the present study suggest with cocaine. This also seems to support the hypothesis, previously posed by Van der Kam et al. (2009a; 2009b), that MPEP reduces the self-administration of addictive drugs because of its reinforcing action.

In conclusion, our results have shown that not only did MPEP not reduce the associative effects of cocaine with environment cues in CPP, but it actually increased them. This potentiating effect is not limited only to cocaine, since seems to affect other drugs of abuse with different mechanisms (Rutten et al., 2011), as well as being able to induce CPP by itself (Van der Kam et al., 2009b). Therefore, these findings do not support the use of mGluR5 negative allosteric modulators, or at the very least MPEP, for the treatment of cocaine relapse.



Acknowledgements

We wish to thank to Guillermo Chuliá for his editing of the manuscript. This work was supported by the following research grants: Ministerio de Economía y Competitividad (MINECO), Dirección General de Investigación, PSI2014-51847-R, PSICO2015-69649-R; Instituto de Salud Carlos III, Red de Trastornos Adictivos (RTA) RD16/0017/0007 and Unión Europea, Fondos FEDER “una manera de hacer Europa”.

Conflict of interests

The authors have no conflicts of interest to disclose.

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