Caffeine, a common active adulterant of cocaine, enhances the reinforcing effect of cocaine and its motivational value.*

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Abstract

Rationale Caffeine is one of the psychoactive substances most widely used as adulterant in illicit drugs, such as cocaine. Animal studies have demonstrated that caffeine is able to potentiate several cocaine actions, although the enhancement of the cocaine reinforcing property by caffeine is less reported, and the results depend on the paradigms and experimental protocols used.

Objectives We examined the ability of caffeine to enhance the motivational and rewarding properties of cocaine using the intravenous self-administration paradigm in rats. Additionally, the role of caffeine as a primer cue during extinction was evaluated.

Methods In naïve rats was assessed: 1) the ability of the combination of cocaine (0.250 - 0.125 mg/kg/infusion) and caffeine (0.125 - 0.0625 mg/kg/infusion) to maintain self-administration in fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement compared with cocaine or caffeine alone; 2) the effect of caffeine (0.0625 mg/kg/infusion) in the maintenance of responding in the animals exposed to the combination of the drugs during cocaine extinction.

Results The combination of cocaine and caffeine and cocaine alone were self-administered on a FR and PR schedules of reinforcement. Interestingly, the breaking point determined for the cocaine and caffeine group was significantly higher than cocaine group. Moreover, caffeine, that per se did not maintain self-administration behavior in naïve rats, maintained drug-seeking behavior of rats previously exposed to combinations of cocaine and caffeine.

Conclusions Caffeine enhances the reinforcing effects of cocaine and its motivational value. Our results highlight the role of active adulterants commonly used in cocaine-based illicit street drugs.

Keywords Reinforcement, Motivation, Addiction, Cocaine, Caffeine, Adulterants, Illicit drugs market.

Introduction

It is well known that illicit drugs of abuse are usually sold in the street with other substances in combination with the main psychoactive ingredient. These substances could be adulterants, impurities or diluents. Adulterant refers to pharmacologically active ingredients while diluents refer to inert substances. Several forensic studies have reported that caffeine is one of the most common psychoactive adulterant found in illicit drugs of abuse (Cole et al. 2011) such as cocaine, either in its snorted (hydrochloride) or smoked forms (coca-paste or crack) (Evrard et al. 2010; López-Hill et al. 2011; Prieto et al. 2015). Caffeine is believed to be added to increase the weight and volume but also to mimic or potentiate the psychostimulant and reinforcing effects of cocaine. However, there are controversial data about caffeine action on the last property (Cole et al 2010).

Some authors consider caffeine as an atypical drug of dependence (Daly and Fredholm 1998) since it strictly fulfills some but not all of the DSM-V criteria for substance dependence. Actually, caffeine is a weak reinforcer and there is little evidence showing clinical dependence induced by its oral consumption (Nehlig and Boyet 2000; Strain and Griffiths 1995). Caffeine is commonly consumed on daily basis through different dietary sources like coffee, tea, cola and energy drinks. At low doses, caffeine can produce positive effects on arousal, vigilance and attention, usually devoid of severe consequences. However, after its chronic consumption and at high doses, symptoms of anxiety, nervousness, impaired thinking, sleep disturbance, heart palpitations and stomach irritation can emerge. Moreover, after a withdrawal period, mild symptoms like fatigue, headache, sleepiness, anxiety and irritability can also appear (Fisone et al. 2004; Fredholm et al. 1999; Nehlig 1999). In animal studies, it was demonstrated that caffeine is able to potentiate several effects of cocaine. For example, caffeine potentiates the

motor stimulation induced by cocaine (Misra et al. 1986; Lopez-Hill et al 2011; Prieto et al. 2012) or the expression of cocaine- or amphetamine-elicited sensitization (Cauli et al. 2003; Simola et al. 2006; Prieto et al. 2015). An additive reinforcement effect of caffeine with cocaine was observed in the conditioned place preference paradigm (Bedingfield et al. 1998). Moreover, caffeine pre-exposure has shown to accelerate the acquisition of cocaine self-administration (Horger et al. 1991) and to increase cocaine responding under a fixed ratio schedule (Schenk et al. 1994). Caffeine can also act as a primer for psychoactive drugs, producing the reinstatement of cocaine-taking behavior (Green and Schenk 2002; Schenk et al. 1996; Worley et al. 1994).

The influence of caffeine on the abuse liability of other stimulants is also reported in clinical studies. In human laboratory studies, intravenous caffeine increased the rate of positive subjective effects in subjects with previous history of drug abuse of cocaine (Rush et al, 1995; Garrett and Griffiths, 2001). It has also been shown that caffeine administration potentiated nicotine effects (Jones and Griffiths, 2003; Perkins et al, 1994). In addition, there is a growing consumption of high caffeinated products like energy drinks (Vester 2014), often in combination with other drugs (Reissig et al. 2009). This evidence raises concerns about the role of caffeine in the development of dependence on other substances or relapse in former drug addicts. The combination with other stimulants and its potential toxicity leads to acute and long term adverse consequences (Derlet et al, 1992; McNamara et al, 2006; Camarasa et al, 2006) which may become aggravated by multiple factors, including the route of administration, chronicity, etc… (Johnson et al, 2010; Kuzmin et al, 2000).

Given these premises, the purpose of this study was to further investigate the role of intravenous caffeine on the rewarding effect of cocaine and its motivational value in the rat. In this regard, we examined the ability of the combination of cocaine and caffeine to

maintain intravenous self-administration in rats under a fixed ratio and a progressive ratio schedule of reinforcement compared to cocaine alone. In addition, the role of caffeine as a primer cue during extinction was evaluated. Also, here we considered a doses ratio found in street samples of the drug of abuse (López Hill et al. 2011; Prieto et al. 2015). To our knowledge this is the first study that investigates the effect of caffeine on the rewarding properties of cocaine under a progressive ratio schedule of reinforcement and in a condition where caffeine is co-administered with cocaine. The results of this approach would help to further understand how the consume of caffeine in combination with other drugs may provoke changes in the abuse liability of these substances.

Methods

Subjects

Male Sprague-Dawley rats (Harlan, Italy), weighing 225-250 g at the beginning of experimental procedures, were housed four per cage with ad libitum food and water, and with constant light–dark cycle (on 7:00 a.m., off 07:00 p.m.), temperature (22°C), and humidity (60%). After surgery, rats were individually housed in plastic cages with ad libitum food and water. For 7-10 days before surgery, rats were handled twice a day. Self-administration (SA) sessions were performed during the light phase, between 9:00 a.m. and 5:00 p.m. After the experimental sessions, the rats were returned to their home cages, where a daily ration of 20 g of food was available. All procedures and experiments were carried out in an animal facility according to Italian (D.L. 116/92 and 152/06) and European Council directives (609/86 and 63/2010) and in compliance with

the approved animal policies by the Ethical Committee for Animal Experiments (CESA, University of Cagliari) and the Italian Department of Health. All efforts have been made to minimize suffering and the numbers of animals used.

Drugs and doses

Cocaine hydrochloride (McFarlan, UK) and Caffeine (free base form, anhydrous, Tocris, UK) were dissolved in sterile saline (0.9 %). Both drugs were given intravenously in a volume of 24 µl per infusion. In order to evaluate the effect induced by caffeine as an active adulterant of cocaine, cocaine and caffeine doses of 0.25 or 0.125 mg/kg, and 0.125 or 0.0625 mg/kg respectively were used. Cocaine doses were selected upon previous data (Valentini et al. 2013). Caffeine doses were selected to keep a similar dose ratio between the drugs found in coca-paste seized samples (López-Hill et al. 2011; Prieto et al. 2015) or in other street samples as reported in literature (see an overview at http://www.drugsandalcohol.ie/13119/).

Catheter implant

Rats were anaesthetized with Equitesin (0.97 g pentobarbital, 4.25 g chloral hydrate, 2.1 g MgSO4, 42.8 ml propylene glycol, 11.5 ml 90 % ethanol/100 ml; 5 ml/kg i.p.) and implanted into the right jugular vein with a catheter (Medical-grade tubing; Silastic, Dow Corning Corporation, Midland, MI, USA) fixed in the middle scapular region by a polypropylene mesh (Evolution, BULEV, weight 48 g/mq, Dipromed, Italy). This ensured stable fixation, rapid tissue integration and reduced foreign body reaction. During recovery, catheters were daily flushed with 0.1 ml of enrofloxacin (50 mg/ml)

and heparinized saline (heparin 250 U/ml in 0.9 % sterile saline). One week after recovery from surgery, rats were randomly assigned to the following groups: a group trained to self-administer the combination of cocaine + caffeine (coc+caff), a second group trained to self-administer cocaine (coc) and a third group trained to self-administer caffeine (caff).

Self-administration

Daily SA sessions were carried out in sound proof boxes (Coulbourn Instruments, Allentown, NJ, USA), provided with two nosepoke holes, one active and the other inactive. A yellow/green light was placed over the active hole and a red light over the inactive one as discriminative stimuli. The responses performed by each rat on both holes (*nose-pokes*) and the corresponding number of reinforces received were recorded. Prior to each daily session, the jugular catheter was flushed with 0.1 ml of sterile saline, and the rats were placed in the SA box.

Schedule Rats, according to the group they belonged to, were trained to self-administer coc+caff (0.25 + 0.125 mg/kg/24 µl), coc (0.25 mg/kg/24 µl) or caff (0.125 mg/kg/24 µl) under a FR1 schedule for 15 daily sessions. By this time rats of coc+caff and coc groups had reached the criterion of 85 % responses on the active hole and were giving stable responses over the last three sessions. Thus, from the 16^{th} to the 20^{th} session doses of coc and caff were reduced by one-half (0.125 and 0.0625 mg/kg, respectively) under the same schedule FR1 for further 5 daily sessions. Thereafter, the schedule of reinforcing was switched to a progressive ratio (PR) for consecutive 7 daily sessions (days 21^{st}-27^{th}). In the PR sessions the work requirement (nose-poking) needed to receive a single i.v. drug infusion was progressively raised within each test session

according to the following PR 3-4 series (meaning the 3rd dose is reached with 4 nosepokes): 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492 and 603 until the breaking point was reached (see details in Richardson & Roberts 1996). The breaking point was defined as the maximal work load (i.e. number of active nosepokes) completed for the last drug infusion prior to a 1-hour period during which no infusions were obtained by the animal. Maximal duration of a PR session was 5 hr. After achieving 3 consecutive days of stable breaking points, each group of rats underwent to the extinction phase (from 28th to the 38th session). In the first phase of extinction (days 28th-34th) the group coc+caff was still allowed to self-administer caffeine alone (0.0625 mg/kg/24 µl) whereas coc group and caff group were shifted to inject saline. Subsequently, saline was substituted for caffeine also in coc+caff group for further 4 sessions (days 35th-38th). For each session, the number of nosepokes emitted (active and inactive), infusions, drug intake (cocaine and caffeine) and breaking points were calculated.

Statistical analysis

Statistical analysis was carried out by Statistica 6 (Stat Soft Inc, US).

A total of 4 rats were excluded from the experiment due to catheter leakage during cocaine SA. The data recorded from these animals were excluded from analysis. Therefore the size of the analyzed and plotted data was derived from 12 rats for coc+caff group, 9 rats for coc group and 11 rats for caff group. Each phase of SA (FR1, PR3-4 and extinction) was assessed by independent analyses.

Nose poking behavior during each daily cocaine SA session and during extinction was analyzed by three-way ANOVA, with group (coc+caff, coc and caff) and cumulative

nose-pokes (active vs inactive) as between-subject factors, and session as repeated measure. Number of infusions and breaking point were analyzed by two-way ANOVA with repeated measures over sessions and group (ie, coc+caff, coc and caff) as between-subject factor. Where significant effects were obtained, multiple pairwise contrasts by Tukey post hoc test were performed. Significance was set at $P < 0.05$.

The between - groups effects on drug intake for each phase of SA and extinction (cocaine or caffeine) were analyzed with unpaired Student's *t*-test ($P < 0.05$).

Results

Cocaine and caffeine intake during self-administration phases

Table 1 shows the average amount of cocaine or caffeine earned during each phase of self-administration by coc+caff, coc and caff groups. Coc+caff and coc groups did not differ in cocaine intake under FR1 with cocaine 0.25 mg ($P = 0.25$) and cocaine 0.125 mg ($P = 0.19$). Moreover, no differences within groups between the two doses of cocaine under FR1 were found ($P > 0.05$). On the other hand, cocaine intake under the PR was higher in coc+caff group than in coc group ($P = 0.003$). With regard to caffeine intake, analysis revealed that the amount of caffeine earned by coc+caff group was higher than caff group during both phases of FR1 ($P < 0.001$) as well as during the PR ($P < 0.0001$). Moreover, caff group significantly reduced caffeine intake when the dose was reduced by one half (0.0625 mg) under FR1 ($P < 0.001$).

Cocaine and caffeine self-administration behavior

Rats were trained to acquire self-administration of cocaine + caffeine (unit dose 0.25 mg/kg and 0.125 mg/kg respectively, 5 day/week), cocaine alone (unit dose 0.25 mg/kg) or caffeine alone (unit dose 0.125 mg/kg) under FR 1 (1^{st}-15^{th} session). From the 16^{th} session the doses of cocaine and caffeine were reduced by one half. From the 21^{th} to the 27^{th} session, the schedule of reinforcement FR1 was replaced with a PR schedule (PR 3-4) followed by 11 session of extinction. The first 7 days of extinction (28^{th} – 34^{th} session), coc+caff group received caffeine (unit dose 0.0625 mg/kg) whereas coc group and caff group were shifted directly to saline (24 μl/infusion). From the 35^{th} session also coc+caff group received saline (24 μl/infusion).

Figure 1 shows cumulative active (panel A) and inactive (panel B) nosepokes performed by coc+caff, coc and caff groups throughout all the phases of SA and extinction.

Cocaine and caffeine self-administration under a FR1 schedule of reinforcement.

Analysis of data from FR1 phase (1^{st} – 20^{th} session) by three-way ANOVA with group (coc+caff, coc and caff), nosepoke (active and inactive) and session as factors, showed a main effect of group ($F_{2,58} = 15.62$, $P < 0.0001$) nosepoke ($F_{1,58} = 129.21$, $P < 0.0001$) and session ($F_{19,1102} = 17.14$, $P < 0.0001$) and group x session ($F_{38,1102} = 4.91$, $P < 0.0001$), group x nosepoke ($F_{2,58} = 18.87$, $P < 0.0001$) and group x nosepoke x session ($F_{38,1102} = 4.75$, $P < 0.0001$) interactions. The coc+caff rats acquired cocaine SA behavior under FR1 schedule from day 8 and, in order to maintain a stable cocaine intake (Table 1), they further increased active nose poking when the dose was reduced by one half under the same schedule ($P < 0.05$ for active nosepoke compared to inactive, and $P < 0.05$ for active nosepoke compared to session 15^{th}, Tukey post-hoc test). Similarly, coc rats acquired cocaine SA behavior under FR1 schedule from day 10 and further increased active nose poking when the dose was reduced by one half ($P < 0.05$ for active nosepoke compared to inactive, and $P < 0.05$ for active nosepoke compared to session 15^{th}, Tukey post-hoc test). Moreover, responding of both groups in the active nosepoke was significantly higher than caff group ($P < 0.05$, Tukey post-hoc test). On the other hand, rats from caff group did not show any consistent nose poking behavior at both doses of caffeine (0.125 and 0.0625 mg/kg) since the number of responding in the active nosepoke did not significantly differ from the inactive nosepoke ($P > 0.05$).

Cocaine and caffeine self-administration under a PR schedule of reinforcement

Analysis of data obtained under the PR3-4 phase (21^{st} – 27^{th} session) by three-way ANOVA revealed a main effect of group ($F_{2,58} = 12.51$, $P < 0.0001$) nosepoke ($F_{1,58} = 25.22$, $P < 0.0001$) and session ($F_{6,348} = 4.78$, $P < 0.0001$) and group x session ($F_{12,348} = 2.02$, $P < 0.05$), group x nosepoke ($F_{2,58} = 10.01$, $P < 0.0001$) and group x nosepoke x session ($F_{12,348} = 3.05$, $P < 0.05$) interactions. The coc+caff group showed a faster and higher active nose poking under PR schedule as compared to the coc group ($P < 0.05$ for coc+caff group active nosepoke compared to inactive, higher coc+caff group active nose poking compared to coc group from the 21^{st} to the 27^{th} session, $P < 0.05$ Tukey post-hoc test). Rats from caff group did not adjust their behavior according to the increased request of the PR schedule. As shown in the FR1 phase, also under PR schedule, responding of coc+caff and coc groups in the active nosepoke was significantly higher than caff group ($P < 0.05$, Tukey post-hoc test).

Extinction of cocaine and caffeine self-administration under a FR1 schedule of reinforcement

Three-way ANOVA of data obtained from extinction phase (28^{th} – 38^{th} session) showed main effect of group ($F_{2,56} = 18.83$, $P < 0.0001$) nosepoke ($F_{1,56} = 63.30$, $P < 0.0001$) and session ($F_{10,560} = 29.95$, $P < 0.0001$) and group x session ($F_{20,560} = 10.14$, $P < 0.0001$), group x nosepoke ($F_{2,56} = 14.61$, $P < 0.0001$) and group x nosepoke x session ($F_{20,560} = 6.89$, $P < 0.0001$) interactions.

During extinction, rats from coc+caff group (that received caffeine for the first 7 sessions) extinguished SA behavior slower than coc group with a reduction of active nose poking only during the last 4 days when caffeine was substituted with saline ($P < 0.05$ for coc+caff group active nosepoke compared to inactive from the 28^{th} to the 34^{th}

session, higher coc+caff group active nose poking compared to coc group from 28th to the 32nd session, $P < 0.05$ Tukey post-hoc test).

In coc group, substitution of cocaine with saline resulted in a marked fall of responding on the active hole after the third saline session ($P < 0.05$ active nosepoke compared to inactive from the 28th to the 30th session, Tukey post-hoc test). Both coc+caff and coc group showed a higher active nose poking behavior than caff group ($P < 0.05$ Tukey post-hoc test).

Figure 2 shows the pattern of responding for a typical rat from each of the groups (coc+caff, coc and caff) at different sessions of FR1, PR and extinction. Both coc+caff and coc groups showed highly regular response patterns under the FR1 schedule of reinforcement (sessions 12 and 16). When animals were shifted to PR 3-4 schedule, the coc+caff group displayed a strong burst of responding and a longer lasting response rate than coc group; with both groups selectively increasing responding on the active nosepoke (session 26). During the early phase of extinction, coc+caff group still maintained a regular overall response pattern, whereas in coc group responding was progressively reduced and erratic (sessions 28-34). Later, responding patterns became erratic also for coc+caff group, indicative of a full extinction of SA behavior. On the other hand, the response patterns of caff group were low and erratic throughout all the sessions.

Cocaine and caffeine infusion during the self-administration behavior

Figure 3 shows the number of infusions earned by rats of each group (coc+caff, coc and caff) throughout all the phases of SA and extinction.

Analysis of data from FR1 phase (1^{st} – 20^{th} session) by two-way ANOVA with group (coc+caff, coc and caff) and session as factors, showed a main effect of group ($F_{2,29}$ = 25.86, $P < 0.0001$) and session ($F_{19,551}$ = 43.26, $P < 0.0001$) and group x session interaction ($F_{38, 551}$ = 10.38, $P < 0.001$).

In the coc+caff group, the number of infusion was higher respect to caff group from day 8 and further increased when the dose was reduced by one half under the same schedule ($P < 0.05$ for coc+caff group compared to caff group, and $P < 0.05$ for coc+caff group infusion compared to session 15^{th}, Tukey post-hoc test). Similarly, in coc rats the number of infusion was significantly higher compared to caff group from day 10 and further increased when the dose was reduced by one half ($P < 0.05$ for coc group compared to caff group, and $P < 0.05$ for coc group infusion compared to session 15^{th}, Tukey post-hoc test). Moreover, during FR1 no difference in the number of infusions was found between coc+caff and coc groups rats ($P > 0.05$).

Analysis of data obtained from PR phase (21^{st} – 27^{th} session) by two-way ANOVA showed a main effect of group ($F_{2,29}$ = 48.16, $P < 0.0001$) and session ($F_{6,174}$ = 4.02, $P < 0.0001$) and group x session interaction ($F_{12, 174}$ = 3.66, $P < 0.001$). Tukey post-hoc test revealed that the number of infusion was significantly higher in coc+caff group compared to coc group and caff group ($P < 0.05$). On the other hand, the number of infusion earned by coc group was higher than caff group as well ($P < 0.05$).

Two-way ANOVA of data obtained from extinction phase (28^{th} – 38^{th} session) showed main effect of group ($F_{2,27}$ = 19.62, $P < 0.0001$) and session ($F_{10,270}$ = 17.00, $P < 0.0001$) and group x session interaction ($F_{20,270}$ = 6.93, $P < 0.001$). During extinction the number of infusions earned by coc+caff remained higher and decreased more slowly compared to coc group ($P < 0.05$, Tukey post-hoc). Moreover, the number of infusions earned by

coc+caff group and coc group was significantly higher than caff group ($P < 0.05$, Tukey post-hoc).

Breaking point

Behavior during the PR3-4 phase was also evaluated as the response requirement of the final ratio completed (breaking point, Fig. 4). Analysis of the results by Two-way ANOVA revealed a main effect of group ($F_{2,29} = 18.77$, $P < 0.0001$) and session ($F_{6,174} = 6.77$, $P < 0.0001$) and group x session interaction ($F_{12,174} = 2.70$, $P = 0.002$). The breaking point of coc+caff group was consistently higher compared to coc group over the sessions ($P < 0.05$, Tukey post-hoc test). Moreover, the breaking point of coc+caff and coc groups was higher than caff group from the 21st and 24th session, respectively ($P < 0.05$, Tukey post-hoc test).

Discussion

The aim of this work was to investigate the role of caffeine in the reinforcement and particularly in motivational and rewarding properties of cocaine. To our knowledge, this is the first time that the effect of the combination of caffeine and cocaine was investigated in a self-administration paradigm with different schedules of reinforcement (FR and PR) and extinction. Another important aspect is the route of administration used for caffeine (intravenously administered) and the dose ratio of the combination of cocaine and caffeine (2:1) comparable to that found in illicit sample from the street.

In our study, we found that during the acquisition of the self-administration behavior on a FR1 schedule, caffeine did not affect the response for cocaine. In fact, rats exposed to self-administer the combination of cocaine and caffeine acquired and maintained the self-administration behavior similarly to rats administering cocaine alone. As expected, caffeine alone did not induce a self-administration behavior, showing no significant difference between the number of active and inactive nosepokes in any of the protocol phases. This last finding is not surprising and confirms previous data from the literature being the primary reinforcing effect of caffeine difficult to measure in animals (Atkinson and Enslen, 1976; Hoffmeister and Wuttke, 1973; Myers and Izbicki, 2006).

Interestingly our results show that caffeine was able to potentiate cocaine self-administration behavior on a PR schedule of reinforcement. Under this phase, rats of the coc+caff group significantly increased their responding on the active nosepoke, the number of infusions and the breaking point compared to coc group. These effects suggest that caffeine has a significant action on motivational properties of cocaine.

Another important finding is that caffeine prolonged the extinction of

responding in animals with a previous history of combination with cocaine. In the extinction phase, in fact, the only presence of caffeine maintained a higher level of responding in rats that previously administered the combination of cocaine and caffeine compared to the coc group. A putative primary reinforcing effect of caffeine is unlikely. Very few studies have shown a role of caffeine as a primary reinforcer but only under particular conditions, at low doses and with limited or intermittent exposures (Myers and Izbicky, 2006; Simola et al, 2006; Sheppard et al, 2012; Retzbach et al, 2014). In our hands, the capacity of caffeine to maintain a high drug-seeking behavior during extinction suggests that it may serve as a primer of cocaine-related reward circuitry. Thus, caffeine doesn't have reinforcing properties itself, but enhancing the rewarding properties of cocaine, behaves as an effective discriminative stimulus for maintaining the drug-seeking behavior in animals.

One major limitation of the present study is the lack of some control groups that would address different issues. In particular, it would have been interesting to study the behavior under the extinction with saline of rats that previously experienced the combination of cocaine and caffeine or vice-versa the effect of caffeine in those animals that previously experienced cocaine alone. Previous data from the literature showed, in the first day of extinction, a higher number of responding in the lever previously associated to cocaine after acute caffeine exposure compared to saline. Also caffeine was able to reinstate extinguished cocaine taking-behavior but this effect was reduced by repeated exposure to caffeine (Worley et al, 1994; Schenk et al, 1996).

On the other hand, it is tempting to speculate that rats previously exposed to the combination of cocaine and caffeine would even display a higher and longer lasting seeking behavior than coc group based on the evidence (present study) that rats of

coc+caff group under a PR schedule showed a higher rate of responding for cocaine and a greater breaking point compared to rats of coc group. This effect would reflect the expression of the greater craving for cocaine in rats in which repeated exposure to caffeine increased the rewarding and motivational properties of the drug.
However, additional studies should be carried out to verify this hypothesis.

The present results not only agree with previous observations but expand the view that caffeine may behave as a potent reinforcement enhancer. More specifically, in animals, caffeine can amplify cocaine-mediated (Harland et al. 1989; Holloway et al. 1985) and nicotine-mediated discriminative effects (Gasior et al. 2000; 2002), increase operant responding for cocaine (Kuzmin et al. 2000; Schenk et al. 1994), nicotine (Shoaib et al. 1999), alcohol drinking (Kunin et al. 2000) and reinstate cocaine seeking behavior (Green and Schenk, 2002; Regier et al, 2014; Worley et al. 1994). In human laboratory studies, intravenous caffeine increases the rate of positive subjective effects in subjects with previous history of drug abuse of cocaine (Rush et al, 1995; Garrett and Griffiths, 2001). Also, recent studies extended this effect of caffeine to non-drug reinforcement (Sheppard et al. 2012).

One possible explanation for the ability of caffeine to increase responding for cocaine could be related to its psychomotor stimulant properties. Exposure to caffeine increases the psychomotor stimulants effects of amphetamine (Cauli et al. 2003; Palmatier et al. 2003; Simola et al. 2006), nicotine (Celik et al. 2006; Gasior et al. 2000) and cocaine (López-Hill et al 2011; Misra et al. 1986; Prieto et al. 2015). However, this effect doesn't explain our results since the increase in responding of coc+caff group was restricted to the active nosepoke, whereas inactive nosepoke responding remained low all times. Therefore, the ability of caffeine to enhance responding for cocaine on the PR schedule and maintain cocaine seeking behavior on extinction phase cannot be easily

attributed to its motor activating effects. It is more likely that caffeine, when chronically administered with cocaine, becomes a potent discriminative stimulus, enhancing the rewarding properties of cocaine. Caffeine is a psychomotor stimulant and, particularly when administered in low doses, produces many of its behavioral effects via adenosine A1 and A2 receptors blockade, influencing indirectly dopaminergic system (Ferré and Fuxe, 1992; Garrett and Griffiths, 1997).

On the other hand, cocaine blocks the dopamine (DA) transporter in the plasma membrane of striatal DA nerve terminal networks. This mechanism leads to a marked increase of DA transmission and is thought to underlie the rewarding/reinforcing actions of cocaine in humans that lead to drug abuse and addiction (Di Chiara and Imperato, 1988; Kalivas and Volkow, 2005; Koob and Bloom, 1988). On this basis, some author reported that caffeine increased DA in the NAc shell (Solinas et al. 2002) suggesting that it might amplify cocaine mediated effects by facilitating dopamine transmission (Green and Schenk, 2002). However, this effect was only obtained after high doses of caffeine and it could not be corroborated by other authors. Acquas et al. (2002) and De Luca et al. (2007) showed that caffeine did not increase DA in the NAc shell and recently this evidence has been extended to humans (Volkow et al. 2015). Thus caffeine, rather than by increasing DA in the striatum, might enhance post-synaptic DA signaling by increasing D2R levels and/or their affinity (Volkow et al. 2015). It is more likely that caffeine, by its antagonism of A2AR in striatal pathways, would facilitate the adenylate cyclase inhibition induced by DA D2 activation (Ferré, 2008). More recently the same author highlighted the role of the striatal A2A-D2 receptor heteromer as the main target of caffeine by which caffeine potentiates the acute and long-term effects of prototypical psychostimulants (Ferré, 2016).

Several data from literature support this evidence. It has been shown that stimulation of A1AR and A2AR reduces numerous cocaine-related behaviors through their ability to functionally oppose selective dopamine receptor activity (Hack and Christie 2003). The stimulation of A1ARs or A2ARs blocks the expression of cocaine sensitization (Filip et al. 2006; Hobson et al. 2012) and impairs the expression of cocaine-conditioned place preference (Poleszak and Malec 2002). On the other hand, antagonism of either A1AR or A2AR substitutes for cocaine and produces leftward shifts in cocaine discrimination (Justinova et al. 2003). In a self-administration paradigm, A2AR stimulation attenuates acquisition of cocaine self-administration (Knapp et al. 2001), whereas antagonism of A2AR enhances responding for cocaine on a progressive ratio schedule of reinforcement without effect on fixed ratio responding (Doyle et al. 2012; Justinova et al. 2011). Furthermore, stimulation of A2A receptors diminishes brain stimulation reward, whereas blocking adenosine receptors reverses the reward impairment produced by cocaine withdrawal or by an A2A agonist (Baldo et al. 1999). Finally, stimulation of A1ARs and A2ARs suppresses cocaine reinstatement, while blockade of A2AR enhances cocaine seeking behavior (Bachtell and Self, 2009; Hobson et al. 2013; O'Neill et al. 2012; O'Neill et al. 2014).

Moreover, besides acting on adenosine receptors, caffeine interacts with PDE, MAO, AChE, ryanodine receptors and others (Pohanka 2015). Thus, interaction with some of these pathways may also account for a direct or indirect potentiation of cocaine effects. However, there is no evidence on metabolic interactions between cocaine and caffeine. Schenk et al (1994) excluded pharmacokinetic alteration of cocaine by caffeine since they found a more pronounced effect of caffeine with low and sub-threshold doses.

In conclusion, the present findings extend the knowledge on the role of caffeine as a potent enhancer of the reinforcing effects of cocaine and the motivational value of

the drug. Several human and animal studies have investigated the interaction of caffeine with nicotine and alcohol, but the literature regarding the interaction of caffeine with other drugs of abuse (e.g. cocaine and heroin) is still meager. These findings are relevant for the important implications that these motivational effects may have in humans. Caffeine is an important ingredient of energy drinks and one of the worldwide adulterant intentionally added to illicit drugs to enhance or mimic their primary effects, particularly to cocaine, in snorted or any of its smoked forms, and heroin (see Cole et al. 2010 for review). Thus, the combination of caffeine with other drugs may increase the motivation to consume the drug (Kozlowsky et al, 1993; Strain et al, 1994; Swanson et al, 1994; Marczinski 2014) with important implications for public health, as individuals tend to engage in more risky behaviors (Jones and Lejuez 2005; Martin et al, 2008). In addition, the present findings give a hint for further studies and are important in terms of their translational potential. In fact, besides the adverse effects that adulterants, and in particular caffeine, have in term of increased toxicity of the adulterated- compared to the unadulterated-drug (Vanattou-Saifoudine et al, 2012), this study highlight the additional concern to the public health of an increased potentiality of abuse.

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	FR1 (1st-15th sess)		FR1 (16th -20th sess)		PR 3-4	
	Cocaine	Caffeine	Cocaine	Caffeine	Cocaine	Caffeine
Group						
coc+caff	4.9±0.6	2.5±0.3#	6.2±0.4	3.1±0.2#	1.9±0.1*	0.9±0.05#
coc	3.9±0.5	------	5.0±0.8	------	1.3±0.1	------
caff	------	1.0±0.1	------	0.6±0.1§	------	0.2±0.02

Table 1 Average amount (mg/kg, mean ± SEM) of cocaine and caffeine earned during each phase of self-administration by coc+caff, coc and caff groups.

* P < 0.05 vs. coc group; # P < 0.05 vs. caff group; § P < 0.05 vs. FR1 (1st-15th sess).

Figure 1

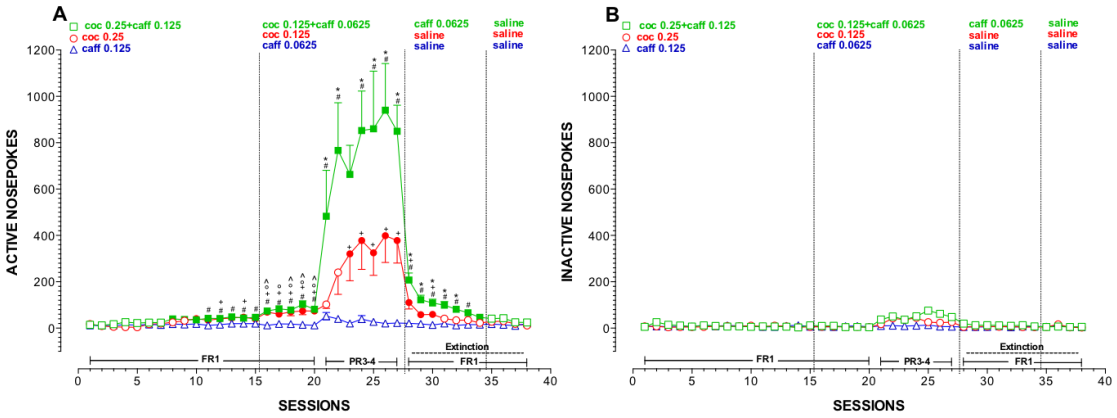

Fig. 1 Cumulative responses during cocaine/caffeine self-administration and extinction (1^{st}–38^{th} session). Results are expressed as mean±SEM of cumulative nosepokes in the active (A) and inactive (B) holes of each group (coc+caff; coc and caff). Filled symbol denotes $P < 0.05$ vs inactive nosepokes; # $P < 0.05$ coc+caff group vs the corresponding active nosepokes of caff group; + $P < 0.05$ coc group vs the corresponding active nosepokes of caff group; ° $P < 0.05$ coc+caff group vs the corresponding active nosepokes of the 15^{th} SA session; ^ $P < 0.05$ coc group vs the corresponding active nosepokes of the 15^{th} SA session; * $P < 0.05$ coc+caff group vs the corresponding active nosepokes of coc group.

Figure 2

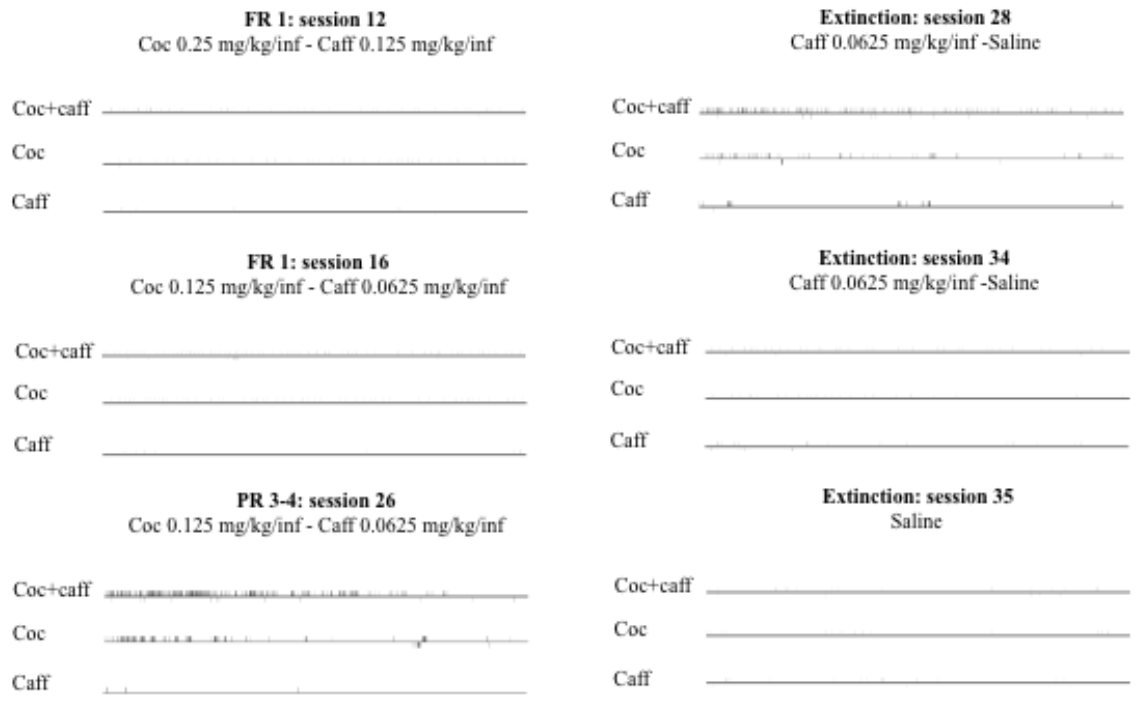

Figure 2 Individual representative records illustrating responding patterns of coc+caff (top trace), coc (middle trace) and caff (bottom trace) rats at the active (upward ticks) and inactive (downward ticks) nosepoke in the sessions throughout the phases of the study. Each tick denotes the time of every nosepoke on the active or inactive lever. During the extinction (sessions 28 and 34) caffeine was available only for coc+caff group.

Figure 3

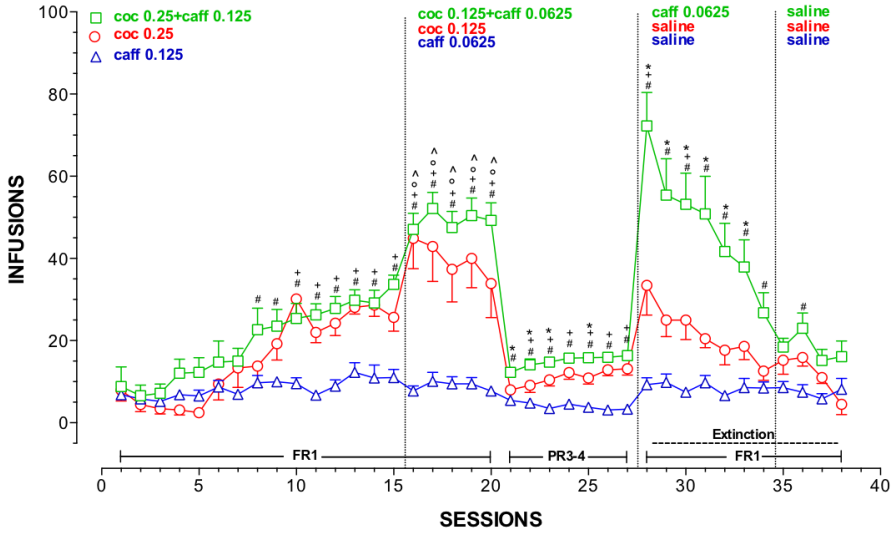

Figure 3 Number of infusions during cocaine/caffeine self administration and during extinction (1^{st}–38^{th} session)**.** Results are expressed as mean±SEM of infusions earned by each group (coc+caff; coc and caff). # P < 0.05 coc+caff group vs the corresponding infusion of caff group; + P < 0.05 coc group vs the corresponding infusion of caff group; ° P < 0.05 coc+caff group vs the 15^{th} SA session; ^ P < 0.05 coc group vs the 15^{th} SA session; * P < 0.05 coc+caff group vs the corresponding infusion of coc group.

Figure 4

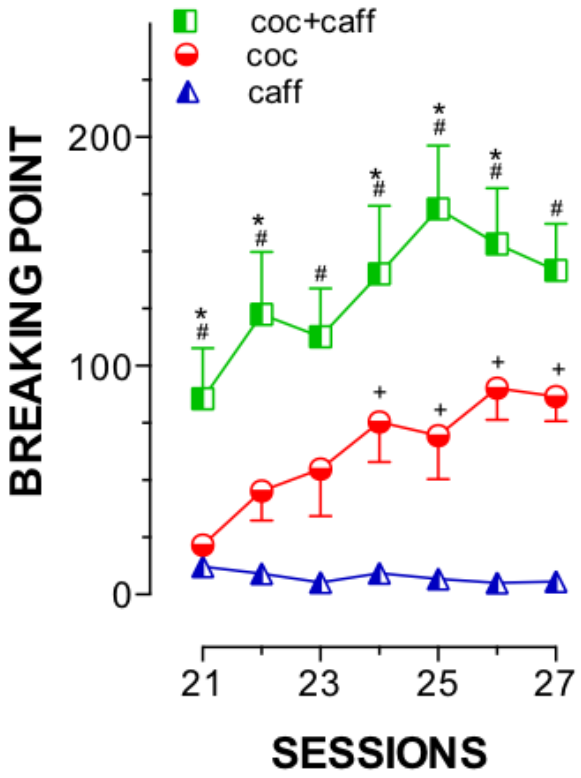

Figure 4 Maximal number of responses (breaking point) completed for the last drug infusion under the PR 3-4 schedule of reinforcement. Results are expressed as mean±SEM of nosepokes emitted by each group (coc+caff; coc and caff). # $P < 0.05$ coc+caff group vs caff group; + $P < 0.05$ coc group vs caff group; * $P < 0.05$ coc+caff group vs coc group.