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ARTICLE

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Influence of acute caffeine on 50-kHz ultrasonic vocalizations in male adult rats and relevance to caffeine-mediated psychopharmacological effects

Nicola Simola[1], Sean T. Ma[2] and Timothy Schallert[2]

[1] *Department of Toxicology, University of Cagliari, Cagliari, Italy*
[2] *Department of Psychology and Institute for Neuroscience, University of Texas at Austin, Austin, TX, USA*

Abstract

To further characterize caffeine-mediated psychopharmacological effects, the present study investigated whether acute caffeine (3, 10, 30, 50 mg/kg i.p.) exerted any influence on the emission and features of ultrasonic vocalizations (USVs), which are thought to index changes involving emotional state, in male adult rats. The results obtained demonstrate that caffeine can trigger modifications in the maximum peak frequency and bandwidth of the 50-kHz range USVs. However, such an effect was not accompanied by a significant elevation in the number of 50-kHz USVs, relative to administration of vehicle. Under the same experimental conditions, acute amphetamine (2 mg/kg i.p.) robustly elevated the number of 50-kHz USVs emitted by rats, although it did not affect the maximum peak frequency and bandwidth of USVs. Thus, both qualitative and quantitative differences in the effects exerted by caffeine and amphetamine on 50-kHz USVs were observed. Taken together, these findings further clarify the features of caffeine-mediated psychopharmacological effects, and may help to elucidate the differences between the central effects of caffeine and those elicited by other psychostimulants.

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Key words: Amphetamine, caffeine, rat, reward, ultrasonic vocalization.

Introduction

Caffeine is one of the most consumed psychoactive substances worldwide and can be found, as either a constituent or an additive, in several dietary sources ranging from coffee, tea and chocolate to the so called 'energy drinks' (Fredholm *et al*. 1999; Mandel, 2002). The popularity of caffeine arises from its ability to elicit psychostimulant and rewarding effects usually devoid of unwanted adverse consequences, which have been described in both experimental animals and humans (Bespalov *et al*. 2006; Haskell *et al*. 2005; Higgins *et al*. 2007; Patkina & Zvartau, 1998; Prediger *et al*. 2005; van Duinen *et al*. 2005).

Previous studies suggest that caffeine-mediated psychopharmacological effects share some similarities with those elicited by psychostimulants bearing abuse potential (Garrett & Griffiths, 1997; Green & Schenk, 2002; Palmatier *et al*. 2005). Moreover, the existence of an additive interaction between the latter substances and caffeine has been demonstrated in rodents by means of different experimental paradigms (Bedingfield *et al*. 1998; Gasior *et al*. 2002; Horger *et al*. 1991; Kuzmin *et al*. 1999). However, in spite of this and additional evidence suggesting that caffeine may target the neuronal circuits involved in addiction (Solinas *et al*. 2002), other studies suggest the existence of major differences between the psychopharmacological effects mediated by caffeine and those elicited by psychostimulants bearing abuse potential (Acquas *et al*. 2002; Bespalov *et al*. 1999; De Luca *et al*. 2007; Kleven & Koek, 1998; Liguori *et al*. 1997), in line with the definition of caffeine as an 'atypical' psychostimulant (Daly & Fredholm, 1998). Based on these considerations, further characterization of the psychopharmacological effects of caffeine is an issue of great interest.

Author for correspondence: Dr N. Simola, Department of Toxicology, Via Ospedale 72, 09124, Cagliari, Italy.
Tel.: +39-070-675-8687 *Fax*: +39-070-675-8612/8665
Email: nicola.simola@virgilio.it

Rats emit ultrasonic vocalizations (USVs) in response to various appetitive and/or aversive conditions, and it has been argued that USVs may reflect the valence and degree of emotional state (Borta *et al*. 2006; Brudzynski, 2005, 2007; Cuomo *et al*. 1992; Knutson *et al*. 2002). USVs are classically categorized into two distinct groups based on their average sound frequency: 22-kHz and 50-kHz vocalizations. In particular, it is believed that rats emit the former in response to aversive stimuli and the latter upon the experience of appetitive/rewarding conditions, although recent findings have suggested that 50-kHz USVs can be emitted also in response to situations which are not necessarily appetitive (Jelen *et al*. 2003; Panksepp & Burgdorf, 2003; Schwarting *et al*. 2007; Wöhr *et al*. 2005, 2008). In this regard, it has been demonstrated that two different types of 50-kHz USVs exist, namely the 'flat' 50-kHz USVs, which display a relatively constant frequency, and the 'trill' (or frequency-modulated) 50-kHz USVs, which are characterized by a variable sound frequency. Recent evidence has suggested that trill USVs may be those more closely indexing the presence of a positive emotional state, whilst flat USVs would mainly function as social-coordinating vocalizations (Wöhr *et al*. 2008).

Several studies have demonstrated that dopamine transmission regulates the emission of USVs by rats (Burgdorf *et al*. 2007; Cagiano *et al*. 1989; Ciucci *et al*. 2007, 2009). Moreover, dopamine critically participates in caffeine-mediated psychopharmacological effects (Borgkvist & Fisone, 2007; Cauli & Morelli, 2005; Ferré *et al*. 1997; Fredholm & Svenningsson, 2003). Finally, previous investigations have shown that amphetamine, methamphetamine and cocaine robustly influence the emission of USVs by rats (Ahrens *et al*. 2009; Armstrong *et al*. 2001; Burgdorf *et al*. 2001; Mu *et al*. 2009; Thompson *et al*. 2006; Wintink & Brudzynski, 2001), thus indicating that USV production is susceptible to psychostimulants bearing abuse potential. Based on these findings, in order to further characterize caffeine-mediated psychopharmacological effects, we evaluated whether acute caffeine affected the acoustic features and number of USVs emitted by adult male rats. The effects mediated by different doses of caffeine (3, 10, 30, 50 mg/kg i.p.) were assessed in separate groups of rats, and the results obtained were compared to the ones elicited by administration of vehicle (i.p.). The effects of caffeine on USVs were also compared to those elicited by acute amphetamine (2 mg/kg i.p.), to reveal the existence of possible similarities and/or discrepancies between the effects mediated by these substances.

Method

Subjects

Adult (aged 6–8 months) Long–Evans male rats were used in this study. Animals were housed in groups of 2–3 in standard polycarbonate cages with sawdust bedding, maintained on a reversed 12-h light/dark cycle (lights off 09:00 hours) and handled daily (5 min) for 3 d before the beginning of the experiments. Food and water were available *ad libitum*, except during the evaluation of USVs, which took place during the dark period of the cycle (from 14:00 to 18:00 hours). All experiments conducted were approved by the University of Texas Animal Care and Use Committee (UT IACUC, protocol number 08040101). Efforts were made to minimize the number of animals used and to maximize humane treatment.

Drugs

Caffeine monohydrate (MP Biomedicals, Germany) was dissolved in warm distilled water and administered i.p. at a dose of 3, 10, 30 or 50 mg/kg. D-amphetamine sulphate (Sigma Aldrich, USA) was dissolved in distilled water and administered i.p. at a dose of 2 mg/kg. The dose of amphetamine used in the present experiments was selected based on a previous study showing that doses of amphetamine in the range of 1.5–2.5 mg/kg (i.p.) stimulate the emission of USVs by adult rats (Wintink & Brudzynski, 2001). An injection volume of 3 ml/kg was used for each drug administration.

USV recording

Recordings of USVs were performed in a sound-isolated Plexiglas chamber (10 cm × 10 cm × 12 cm) equipped with an ultrasonic microphone bearing high directional properties (CM16, Avisoft, Germany). The microphone had a flat frequency response of up to 150 kHz. USV was sampled at 200-kHz sampling rate through an AD/DA card (National Instruments, USA). During all recordings intensity gain remained at the same level for all subjects. Each rat was individually placed into the recording chamber and after a 2-min delay, USVs were recorded for 15 min.

Experimental protocol

Experiments took place over 5 consecutive days. On days 1, 2 and 3 each rat was individually placed in the USV recording chamber and left undisturbed for 15 min. The bottom of the recording chamber was covered in sawdust taken from the home cage of each

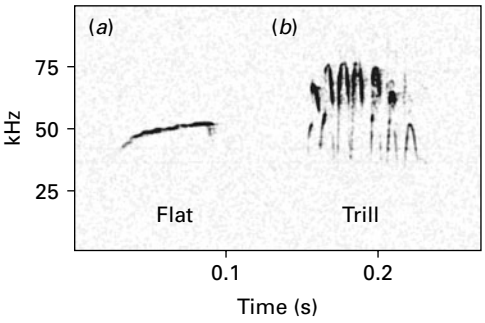

Fig. 1. Exemplificative sonograms of (*a*) flat and (*b*) frequency-modulated trill 50-kHz ultrasonic vocalizations (USVs). Audio samples of 50-kHz USVs (slowed down 20×) can be found at the Schallert laboratory website (www.schallertlab.org).

particular animal tested. This procedure was performed to acclimate the rats to the test environment. On day 4, each rat received an injection of vehicle (i.p.), and was immediately placed into the recording chamber. USVs were then recorded for 15 consecutive minutes, starting 2 min after the rat was placed in the recording chamber. On day 5, to test drug-induced USVs, each rat received an injection (i.p.) of either caffeine (3, 10, 30, 50 mg/kg) or amphetamine (2 mg/kg) and was promptly placed into the recording chamber. USVs emitted by each rat were then recorded for 15 consecutive minutes, following a 2-min delay. The testing chamber was thoroughly cleaned after each rat. The recording of USVs was narrowed to 15 min based on preliminary observations which had demonstrated an almost absent emission of USVs by rats subject to the same experimental protocol in response to either vehicle (i.p.) or caffeine (3, 10, 30 mg/kg i.p.) as measured between 30 and 50 min from injection (N. Simola, unpublished observations). For each group of animals receiving either caffeine or amphetamine, $n=7$ rats.

Data analysis and statistics

USV recordings were collected on a computer and transferred to an external hard drive for storage and analysis. Recorded USVs were analysed with Saslab Pro (Avisoft), which automatically calculates the number of USVs and acoustic parameters for each USV. Sonograms were generated under a 512 FFT-length and 75% overlap frame set-up. Individual USVs were then separated into single WAV files for further analysis. USVs were categorized into 'flats' or 'trills' (frequency modulated) based on their structure by an experimenter unaware of the animals' treatment (Fig. 1). In addition, a further classification of USVs into flats or trills was performed based on their bandwidth (calculated as the maximum peak frequency minus the minimum peak frequency in each USV) according to the criteria proposed by Wang and colleagues (Wang *et al*. 2008). Hence, USVs displaying a bandwidth $\leqslant$5000 Hz were classified as flats, whereas USVs displaying a bandwidth $\geqslant$5000 Hz were classified as trills. The results obtained by using the latter procedure overlapped those obtained after visual categorization. Therefore, for more clarity and to ensure the replication of the results, bandwidth-based classification was chosen as the referring methodology for further statistical analysis.

The number of flat, trill and total (flat + trill) USVs, were scored for each individual rat and the means ± S.E.M. of USV totals were then calculated for each experimental group. Data obtained after the administration of vehicle were pooled together. To characterize the influence of each pharmacological treatment on the qualitative features of rats' vocalization, individual USVs were pooled together for each treatment group. Then average maximum peak frequency, bandwidth and duration of flat and trill USVs were calculated together with the ratio between trill and flat USVs. The presence of significant between-group differences was evaluated by means of one-way ANOVA followed by Tukey's HSD *post-hoc* test. Statistical analysis was performed with SPSS for Windows (SPSS Inc., USA).

Results

General considerations

In the present study, the emission of 22-kHz USVs was observed only in one rat that had received caffeine at a dose of 50 mg/kg i.p., and this rat made only eight USVs in 15 min. Therefore, only USVs belonging to the 50-kHz category will be discussed further.

The administration of caffeine was found to dose-dependently affect both the peak frequency and the bandwidth of USVs, as well as composition of USVs by shifting the relative proportion between trill and flat USVs. Administration of caffeine was also associated with a trend towards an increase in the number of USVs emitted, although this effect did not reach statistical significance. Finally, both qualitative and quantitative differences in the effects exerted by caffeine on USV emission were observed in regard to those elicited by amphetamine. For more clarity, modifications in acoustic parameters are shown in the Figures as percentage change relative to the vehicle group. Raw numeric data are reported in Table 1.

Table 1. Modifications in the acoustic parameters of flat and trill 50-kHz ultrasonic vocalizations observed after the administration of caffeine or amphetamine

Treatment	PF flat	PF trill	BW flat	BW trill
Vehicle	54765.3 ± 400	67462.3 ± 744	2448.8 ± 67	17443.4 ± 692
Caffeine (3 mg/kg)	53416.3 ± 363	61938.4 ± 447*#	2512.3 ± 67	12097.3 ± 420*
Caffeine (10 mg/kg)	57039.7 ± 909	72714.3 ± 2414	1974.1 ± 173	23311.9 ± 2766*#
Caffeine (30 mg/kg)	56708.3 ± 580	69658.3 ± 619	2602.3 ± 117	18545.8 ± 641
Caffeine (50 mg/kg)	48751.1 ± 356*	60130 ± 751*#	2111 ± 69*	17263 ± 729
Amphetamine (2 mg/kg)	55773.9 ± 251	67599.3 ± 258	2443.7 ± 48	18108.4 ± 266

PF, Maximum peak frequency; BW, bandwidth.
All the values reported are expressed in Hz.
* $p < 0.05$ relative to the correspondent vehicle group. # $p < 0.05$ compared to the correspondent amphetamine group.

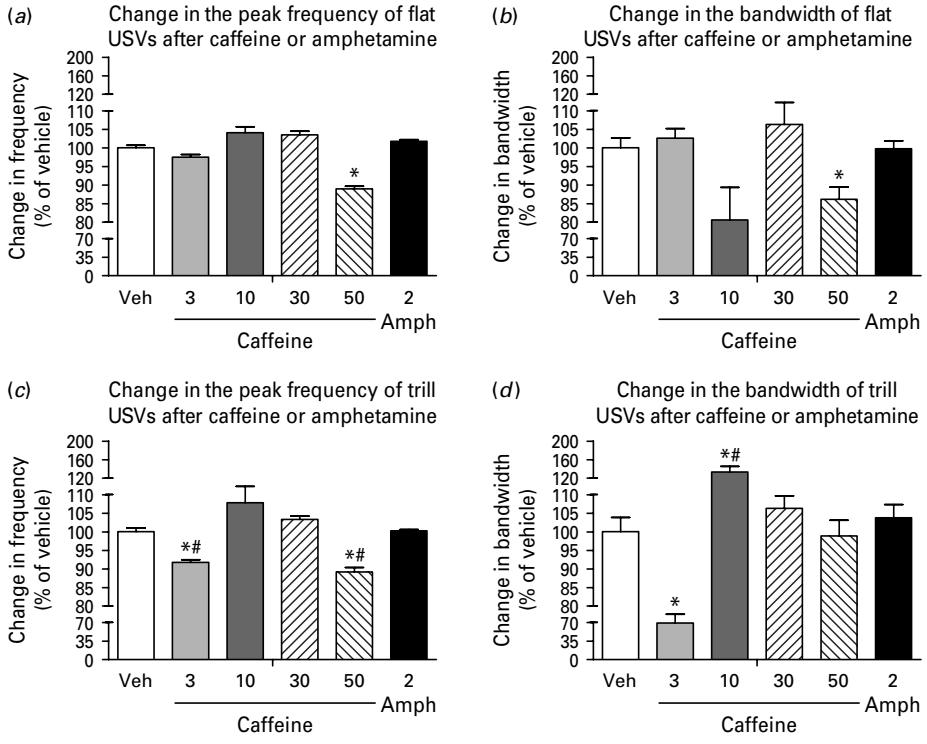

Fig. 2. Changes in the acoustic parameters of flat and trill (frequency-modulated) 50-kHz ultrasonic vocalizations (USVs) triggered in rats by the administration of vehicle (i.p.), caffeine (3, 10, 30, 50 mg/kg i.p.) or amphetamine (2 mg/kg i.p.). Panels (*a*) and (*b*) display the modifications in the peak frequency and bandwidth of flat USVs. Changes in the peak frequency and bandwidth of trill USVs are shown in panels (*c*) and (*d*). Modifications in acoustic parameters of USVs are expressed as percentage of vehicle group. * $p < 0.05$ compared to the administration of vehicle. # $p < 0.05$ compared to the administration of amphetamine. $n = 58$–819 for flat USVs. $n = 42$–1748 for trill USVs. $n = 7$ rats for each dose of caffeine and for amphetamine. $n = 35$ rats for vehicle.

Effects on the peak frequency of USVs

A significant effect of the pharmacological treatment on the maximum peak frequency was observed for flat ($F = 55.94$, $p < 0.01$) and trill ($F = 38.38$, $p < 0.01$) USVs. Tukey's *post-hoc* test revealed specific effects of caffeine, but not amphetamine, on this acoustic parameter (Fig. 2). Thus, the comparison between caffeine- and vehicle-stimulated USVs revealed that: (i) caffeine (3 mg/kg i.p.) caused a significant reduction in the

maximum peak frequency of trill ($p<0.01$), but not flat, USVs (Fig. 2*a*,*c*); (ii) caffeine (10 mg/kg i.p.) exerted no significant influence on the maximum peak frequency of flat and trill USVs, although an overall trend towards an increase in this acoustic parameter was observed (Fig. 2*a*,*c*); (iii) caffeine (30 mg/kg i.p.) did not cause any significant changes in the maximum peak frequency of either flat or trill USVs (Fig. 2*a*,*c*); (iv) caffeine (50 mg/kg i.p.) elicited a reduction in USV maximum peak frequency of both flat ($p<0.01$) and trill ($p<0.01$) USVs. Under the same experimental conditions, acute amphetamine (2 mg/kg i.p.) did not affect the maximum peak frequency of either flat or trill USVs (Fig. 2*a*,*c*).

Effects on the bandwidth of USVs

A significant effect of the pharmacological treatment was also observed on the bandwidth of flat ($F=55.94$, $p<0.01$) and trill ($F=38.38$, $p<0.01$) USVs. Tukey's *post-hoc* test revealed that caffeine, but not amphetamine, affected USV bandwidth (Fig. 2). When the caffeine-stimulated USVs were compared to the vehicle-elicited ones, it was observed that: (i) caffeine (3 mg/kg i.p.) caused a significant reduction in the bandwidth of trill ($p<0.01$), but not flat, USVs (Fig. 2*b*,*d*); (ii) caffeine (10 mg/kg i.p.) led to a significant increase in the bandwidth of trill USVs ($p<0.02$), which was accompanied by an almost significant decrease in the bandwidth of flat USVs ($p=0.08$) (Fig. 2*b*,*d*); (iii) caffeine (30 mg/kg i.p.) did not elicit modifications in the bandwidth of either flat or trill USVs (Fig. 2*b*,*d*); (iv) caffeine (50 mg/kg i.p.) caused a reduction in the bandwidth of flat USVs ($p=0.01$), but not of trill USVs (Fig. 2*b*,*d*). Under the same experimental conditions, acute amphetamine (2 mg/kg i.p.) did not exert any influence on the bandwidth of flat and trill USVs (Fig. 2*b*,*d*).

Effects on the number of USVs, trill/flat ratio and duration of USVs

In line with the results obtained on acoustic parameters of USVs, a significant effect of the pharmacological treatment was observed when the number of flat ($F=5.3$, $p<0.01$), trill ($F=4.45$, $p<0.01$) and total (flat + trill) ($F=12.6$, $p<0.01$) USVs were analysed (Fig. 3*a*–*c*). Tukey's *post-hoc* test revealed that administration of amphetamine (2 mg/kg i.p.) significantly increased the number of USVs emitted, relative to vehicle (i.p.). Conversely, Tukey's *post-hoc* test indicated that there was no significant increase in the number of USVs with caffeine administration (Fig. 3*a*–*c*). Nevertheless, a dose-dependent trend towards an increase in this parameter was observed in response to caffeine (Fig. 3*a*–*c*). Finally, Tukey's *post-hoc* test showed that amphetamine (2 mg/kg i.p.) was significantly more potent than any dose of caffeine tested in elevating the number of USVs.

A significant effect of the pharmacological treatment was also observed on the ratio between trill and flat USVs ($F=3.04$, $p<0.05$) (Fig. 3*d*). Tukey's *post-hoc* test revealed a significant increase of this parameter subsequent to the administration of amphetamine (2 mg/kg i.p.). On the other hand, caffeine administration did not yield significant changes in the trill/flat ratio, although it was found increased after 30 mg/kg caffeine and lowered after 3, 10 and 50 mg/kg caffeine, compared to vehicle (Fig. 3*d*).

Neither dose of caffeine tested in this study, nor amphetamine (2 mg/kg i.p.), elicited significant modifications in duration of USVs, with regard to vehicle (data not shown).

Discussion

This study reports, for the first time, the effects exerted by caffeine on the emission of USVs in adult rats. The results obtained demonstrate that 50-kHz USVs emitted by rats in response to an acute administration of caffeine displayed different features compared to 50-kHz USVs stimulated by an administration of vehicle. In fact, caffeine triggered changes involving the bandwidth and peak frequency of USVs. However, caffeine administration did not cause a significant increase in the number of USVs. Furthermore, major differences were found between the effects on 50-kHz USVs emission mediated by caffeine and those exerted by amphetamine. Thus, amphetamine greatly augmented the number of USVs emitted and substantially increased the ratio between the trill and flat type of USVs, but it did not affect the acoustic parameters of USVs compared to caffeine or vehicle.

In the present study, caffeine influenced both the peak frequency and the bandwidth of 50-kHz USVs. Notably, a marked dose-dependence of the effects of caffeine on the acoustic parameters of 50-kHz USVs was observed. In particular, caffeine selectively affected the acoustic parameters of the trill type of USVs when administered at a dose of 3 mg/kg. Such selective an effect was not present at increasing doses, as either modifications in the acoustic parameters of both trill and flat USVs or no effect was observed. Thus, administration of 10 mg/kg caffeine yielded reciprocal changes in the bandwidth of 50-kHz USVs, with the bandwidth of the trill USVs being increased and that of the flat USVs being decreased, although not

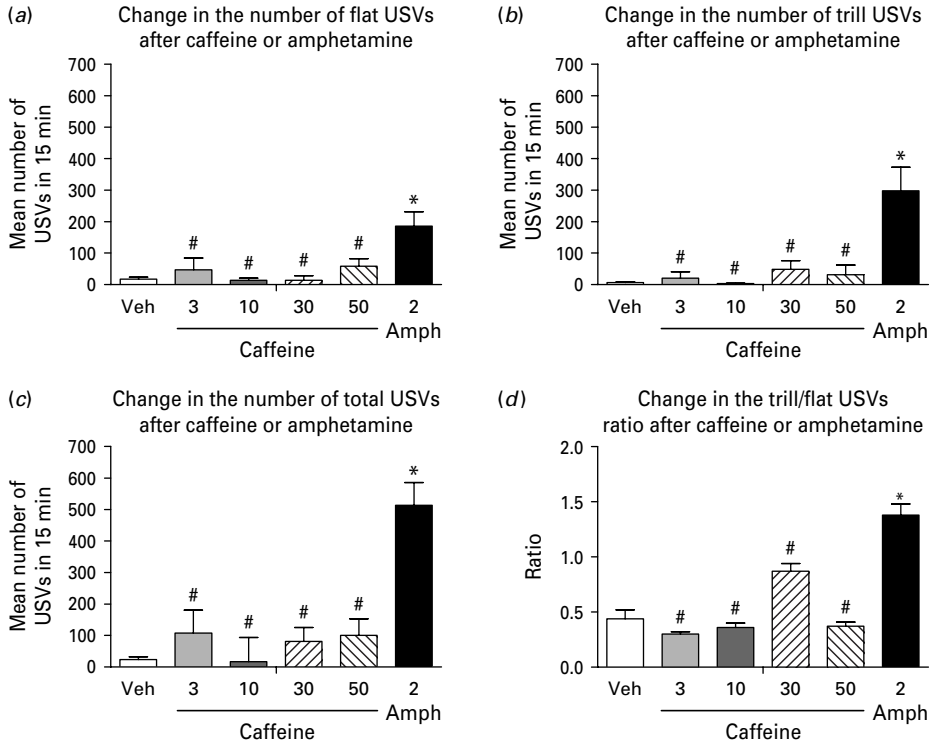

Fig. 3. Effect of vehicle (i.p.), caffeine (3, 10, 30, 50 mg/kg i.p.) or amphetamine (2 mg/kg i.p.) on the number of flat, trill and total (flat + trill) 50-kHz ultrasonic vocalizations (USVs) emitted by rats and on the ratio between trill and flat USVs. The number of USVs represents the average number of vocalizations recorded during 15 min of experiment. Amphetamine (2 mg/kg i.p.), but not caffeine, led to a significant increase in the number of (*a*) flat, (*b*) trill and (*c*) total (flat + trill) USVs as well as in the ratio between trill and flat USVs (*d*). * $p < 0.05$ compared to the administration of vehicle. # $p < 0.05$ compared to the administration of amphetamine. $n = 7$ for each dose of caffeine and for amphetamine. $n = 35$ for vehicle.

significantly for the latter. Moreover, this dose of caffeine resulted in a trend towards an increase in the peak frequency of both trill and flat USVs. The effects of caffeine on acoustic parameters of 50-kHz USVs appeared to be lost at the higher dose of 30 mg/kg, whilst a generalized depression of the acoustic parameters of 50-kHz USVs was observed after 50 mg/kg caffeine administration. Such a depression involved the peak frequency of trill and flat USVs and the bandwidth of flat USVs. However, in this regard it should be considered that very high doses of caffeine exert effects which often differ from those observed at lower doses (Fredholm *et al*. 1999). The relevance of the results observed after 50 mg/kg caffeine to caffeine-mediated psychostimulant effect should therefore be interpreted cautiously. Conversely to the results obtained after administration of caffeine at moderate-to-high doses, when administered at the dose of 3 mg/kg, it affected both the peak frequency and bandwidth of trill USVs but did not lead to any changes, or to trends towards a change, in the acoustic parameters of flat USVs, which were found to be very similar to those observed after vehicle administration. This latter finding appears particularly interesting, as the trill 50-kHz rather than the flat USVs, have been suggested to be a behavioural correlate of a 'positive' emotional state in rats (Wöhr *et al*. 2008). Interestingly, Brudzynski (2005) has suggested that changes in the peak frequency reflect, in some instances, the emotional value of 50-kHz USVs. Furthermore, previous studies have demonstrated that doses of caffeine in the range of 1–30 mg/kg affect conditioned place preference in the rat, which is a behaviour that measures the presence of rewarding/aversive properties of psychoactive substances (Brockwell *et al*. 1991; Patkina & Zvartau, 1998; Tzschentke, 2007). In particular, doses of caffeine in the range of 1–3 mg/kg have been demonstrated to bear rewarding properties, whilst higher doses have been shown to be aversive. Therefore, it can be hypothesized that the modifications in the peak frequency of trill USVs triggered by the administration of a low dose of caffeine might be

a correlate of the effects exerted by caffeine on the neuronal systems governing reward. However, further studies are necessary to address this hypothesis, and to elucidate the relative relevance to reward processes of modifications in acoustic parameters of 50-kHz USVs *vs*. changes in the number of 50-kHz USVs. In fact, next to changes in peak frequency, the modification in the number of vocalizations emitted has been indicated as another major parameter indexing the emotional value of 50-kHz USVs (Brudzynski, 2005) and in the present study caffeine had no effect on the number of USVs emitted at any dose tested (see below).

The comparative analysis of the qualitative features of the USVs stimulated by caffeine and amphetamine revealed both differences and similarities. Thus, as previously described, caffeine was found to trigger modifications in the acoustic parameters of trill and flat USVs. Conversely, amphetamine was observed not to affect the acoustic parameters of either type of USV. However, amphetamine administration affected the composition of USVs, as it led to a robust increase in the ratio between trill and flat USVs. Interestingly, the absence of changes in the acoustic parameters of either trill or flat USVs associated with a trend towards an increase in the trill/flat ratio were also observed in response to administration of 30 mg/kg caffeine. Taken together, these findings point, on the one hand, to the existence of complex effects of caffeine on USV emission. On the other hand, they indicate that caffeine, at least at moderately high doses, may influence the emission of USVs in a fashion resembling the one elicited by a psychostimulant bearing addiction potential. Additional studies employing a wide range of amphetamine doses appear necessary to further address the qualitative discrepancies and similarities between the effects of caffeine and amphetamine on USVs evidenced by this study.

When the quantitative features of caffeine-stimulated USVs were analysed, it was observed that caffeine did not significantly increase the number of USVs relative to vehicle. Furthermore, the number of caffeine-stimulated USVs after any dose tested was markedly lower than the number observed after amphetamine administration. This latter finding clearly indicates that caffeine is less potent than amphetamine in stimulating the emission of USVs in rats. Interestingly, the number of USVs has been suggested as another parameter, together with peak frequency, that reflects the emotional value of 50-kHz USVs (Brudzynski, 2005). A recent study in rats repeatedly administered amphetamine or cocaine on alternate days has shown the manifestation of a progressive increase in the number of 50-kHz USVs associated with the development of sensitization to the motor effects of those psychostimulants (Ahrens *et al*. 2009; Mu *et al*. 2009). Notably, the development of psychomotor sensitization has been suggested by previous studies to reflect the occurrence of neuroplastic modifications in brain areas regulating reinforcement (Robinson & Berridge, 2000; Vanderschuren & Kalivas, 2000). Therefore, it can be argued that the marginal increase in the number of 50-kHz USVs stimulated by caffeine observed in the present study is a consequence of the relatively weak reinforcing properties of caffeine, which have been demonstrated by several studies, in line with the definition of caffeine as an 'atypical psychostimulant' (Daly & Fredholm, 1998; Griffiths & Chausmer, 2000). Caffeine-stimulated USVs may represent a useful new tool for use in the study of addictive-like properties of caffeine. Indeed, recent investigations have shown that caffeine triggers the development of sensitization to its motor stimulant effects, which are accompanied by enduring neuroadaptive changes in striatal regions (Hsu *et al*. 2009; Simola *et al*. 2006, 2008; Tronci *et al*. 2006). Therefore, addressing whether, and to what extent, such changes are paralleled by modifications in the features and number of USVs may prove valuable.

Regarding the possible mechanisms underlying the ineffectiveness of caffeine in robustly stimulating USVs emission, a major one might involve the specific way caffeine interacts with dopamine transmission. Dopamine transmission modulates the emission of 50-kHz USVs in a fashion that is dependent on specific brain regions (Burgdorf *et al*. 2001; Ciucci *et al*. 2007; Wintink & Brudzynski, 2001). Brain mapping experiments have demonstrated that USVs are substantially increased in response to an infusion of the dopaminergic psychostimulant amphetamine into the nucleus accumbens shell (Thompson *et al*. 2006). Conversely, such an effect was observed to be significantly less intense when amphetamine was infused into the nucleus accumbens core, and to be almost absent when amphetamine was delivered in the caudate putamen nucleus (Burgdorf *et al*. 2001; Thompson *et al*. 2006). Although caffeine is known to boost dopaminergic transmission (Borgkvist & Fisone, 2007; Cauli & Morelli, 2005; Ferré *et al*. 1997; Fredholm & Svenningsson, 2003), no consensus exists on whether, and to what extent, nucleus accumbens shell dopamine would be affected by caffeine. While the ability of caffeine to stimulate dopamine release in the nucleus accumbens shell has been reported (Solinas *et al*. 2002), conversely, other studies have shown no modifications in dopamine levels in this brain region following

caffeine (Acquas *et al*. 2002; De Luca *et al*. 2007). Furthermore, investigations evaluating the dopamine-dependent expression of either the immediate early gene *zif-268* (NGFI-A) or the phospho-protein p-ERK in the nucleus accumbens shell (Svenningsson *et al*. 1997; Tronci *et al*. 2006; Valjent *et al*. 2004; Wang *et al*. 1995) have revealed the existence of major differences between the effects exerted by caffeine and those elicited by either amphetamine or cocaine, which robustly boost dopamine transmission in that region (Di Chiara & Imperato, 1988). The lack of an increase in the number of 50-kHz USVs in response to caffeine administration reported here may therefore depend on the fact that caffeine fails to boost dopamine transmission in the nucleus accumbens shell sufficiently to trigger a robust emission of USVs. However, the role of both dopaminergic and non-dopaminergic mechanisms in caffeine-stimulated USV production should be approached. In fact, the involvement of glutamate and acetylcholine in the modulation of 50-kHz USV emission has been demonstrated (Wang *et al*. 2008; Wintink & Brudzynski, 2001), and the existence of interactions between caffeine and such neurotransmitters is well acknowledged (Fredholm *et al*. 1999).

In conclusion, this study demonstrates that caffeine can influence certain features of 50-kHz USVs, a proposed behavioural correlate of emotional state in adult rats. Furthermore, this study may be relevant to the elucidation of the differences and similarities existing between the effects of caffeine and those of other psychostimulants, proposing in addition, the use of USVs as a new tool for the study of caffeine-mediated psychopharmacological effect.

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Statement of Interest

None.

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