Ultrasonic vocalization in rats self-administering heroin and cocaine in different settings: evidence of substance-specific interactions between drug and setting

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Abstract
Rationale Clinical and preclinical evidence indicates that the setting of drug use affects drug reward in a substance-specific manner. Heroin and cocaine co-abusers, for example, indicated distinct settings for the two drugs: heroin being used preferentially at home and cocaine preferentially outside the home. Similar results were obtained in rats that were given the opportunity to self-administer intravenously both heroin and cocaine.

Objectives The goal of the present study was to investigate the possibility that the positive affective state induced by cocaine is enhanced when the drug is taken at home relative to a non-home environment, and vice versa for heroin.

Methods To test this hypothesis, we trained male rats to self-administer both heroin and cocaine on alternate days and simultaneously recorded the emission of ultrasonic vocalizations (USVs), as it has been reported that rats emit 50-kHz USVs when exposed to rewarding stimuli, suggesting that these USVs reflect positive affective states.

Results We found that Non-Resident rats emitted more 50-kHz USVs when they self-administered cocaine than when self-administered heroin whereas Resident rats emitted more 50-kHz USVs when self-administering heroin than when self-administering cocaine. Differences in USVs in Non-Resident rats were more pronounced during the first self-administration (SA) session, when the SA chambers were completely novel to them. In contrast, the differences in USVs in Resident rats were more pronounced during the last SA sessions.

Conclusion These findings indicate that the setting of drug taking exerts a substance-specific influence on the ability of drugs to induce positive affective states.

Keywords Ultrasonic vocalizations · USVs · Drug abuse · Cocaine · Heroin · Self-administration · Emotion · Environment · Context · Setting · Reward · Affect

Introduction
Previous experiments have shown that the setting of drug taking exerts a powerful influence on the rewarding effects of heroin and cocaine and that this influence is substance-specific. Cocaine self-administration (SA), for example, is greatly facilitated when rats self-administer the drug in an environment that is distinct from the home environment (Non-Resident rats) relative to rats for whom the SA chamber is also the home environment (Resident rats) (Caprioli et al. 2007a, b). Non-Resident rats also exhibit greater motivation for cocaine SA than Resident rats, as indicated by progressive ratio reinforcement schedule procedures. In contrast, Resident rats self-administer more heroin than Non-Resident rats and also exhibit greater motivation in break-point procedures (Caprioli et al. 2008). Furthermore, Non-Resident rats tend to prefer cocaine to heroin in a choice procedure, whereas Resident rats tend to prefer heroin to cocaine (Caprioli et al. 2009). Finally, Non-Resident rats are more vulnerable to relapse into...
cocaína (en respuesta a priming cocaína) en ratones residentes, mientras que los ratones residentes son más vulnerables a relapser en la búsqueda de cocaína. 

El comportamiento de la búsqueda de cocaína y heroina puede ser influenciado por el contexto, que incluye el entorno doméstico y no-doméstico. Aunque se ha sugerido que los ratones prefieren el entorno doméstico para la búsqueda de heroina (Caprioli et al. 2009), se ha demostrado que los adictos prefieren el entorno doméstico para la búsqueda de cocaína, independientemente del tipo de entorno (Montanari et al. 2015). Estos efectos moduladores del contexto no son únicos a los ratones de laboratorio, sino que también se observan en humanos.

La aparente disparidad en el comportamiento de la búsqueda de cocaína y heroina se puede explicar mediante el principio de la “desviación” entre el contexto y el estado interno del organismo. De acuerdo con esta hipótesis, el centro y los efectos periféricos (samilpsomímicos) del consumo de cocaína se evaluarán como menos aterrador en comparación con el entorno no-doméstico, mientras que el cocaína sería aterrador en el entorno doméstico. De esta manera, el efecto de las drogas se reduce según este supuesto. Según esta hipótesis, el centro y los efectos periféricos (samilpsomímicos) del consumo de cocaína se evaluarán como menos aterrador en comparación con el entorno no-doméstico, mientras que el cocaína sería aterrador en el entorno doméstico. De esta manera, el efecto de las drogas se reduce según este supuesto.

En el presente estudio, se utilizan vocalizaciones ultrasonicas (USVs) emitidas por ratones auto-administrando cocaína y cocaína, como un índice de los estados afectivos del ratón. De hecho, las USVs de 50-kHz se emiten en respuesta a estímulos recompensantes (Knutson et al. 1998), heterospecíficos (Burgdorf y Panksepp 2001; Målo y cols. 2007; Panksepp y Burgdorf 2000, 2003; Schwarting y cols. 2007; Wörh et cols. 2009), sexo (McGinnis y Vakulenko 2003; White et cols. 1990; Bialy et cols. 2000), comida (Burgdorf et cols. 2000), estimulación eléctrica (Knutson et cols. 1999; Schwarting y cols. 2010; Wintink y Brudzynski 2001; Wright et cols. 2010; Barker et cols. 2010; Maier et cols. 2010). En contraste, las USVs de 22-kHz son emitidas en asociación con la exposición a estímulos aterrador, como la electric shock (Lee et cols. 2001; Koo et cols. 2004) y los depredadores (Blanchard et cols. 1991, 1992), la retirada de cocaína (Covington y Miczek 2003; Mutschler y Miczek 1998; Vivian y Miczek 1991), la defensiva o los estados perturbadores durante el comportamiento agresivo (Lore et cols. 1976; Portavella et cols. 1993; Thomas et cols. 1983), y el dolor crónico (Calvino et cols. 1996). Asimismo, se ha propuesto que las USVs de 22-kHz reflejan los estados afectivos internos negativos de la rata, mientras que las USVs de 50-kHz reflejan los estados afectivos internos positivos (Barker 2010).

**Materials and methods**

**Animals**

Un total de 32 ratones machos Sprague-Dawley (Harlan Laboratories) de un peso de 250–280 g fueron utilizados. Catorce ratones fueron excluidos de la analítica debido a que no lograron alcanzar el criterio de SA (al menos dos infusiones por sesión durante las seis últimas sesiones de SA). Un ratón murió durante el experimento. Los ratones fueron alojados y probados en el mismo ambiente controlado de temperatura y humedad (21 ± 1 °C; 70 %), con acceso libre (excepto durante las sesiones de prueba) a alimentos y agua durante una vuelta de 14 h de luz/10 h de oscuridad (a las 7:00 a.m. en el a.m.). Los ratones fueron manejados gentilmente dos veces a la semana durante 2 semanas antes de someterlos a la cirugía de implantación.

**Catheter surgery**

En el día de la cirugía, los ratones recibieron una inyección i.p. de 2.33 mg de la solución salina que contiene 0.4 mg/l de enrofloxacino (Baytril®, KVP Pharma + Veterinär Produkte Gmbh, Kiel, Germany). Las cátaros fueron flujadas diariamente con 0.1 ml de una solución salina esterilizada que contiene 0.4 mg de enrofloxacino en 25 IU heparina (Marvecs Services, Agrate Brianza, Italy).

**Self-administration procedures**

Después del día de la cirugía, los ratones fueron asignados al grupo Resident o Non-Resident. Los ratones residentes fueron alojados en la cámara de SA durante todo el experimento, mientras que los residentes no fueron alojados en cajas de polycarbonato estándar y fueron transferidos a las cámaras de SA solo para las sesiones de SA (para más detalles sobre el aparato y el alojamiento, vea Caprioli et al. 2007a). Los cátaros de los ratones residentes fueron conectados a las líneas de infusión 3 h antes del inicio de la SA. Los ratones fueron entrenados para auto-administrar cocaína (400 μg/kg por infusión) y heroina (25 μg/kg por infusión) en turnos alternados por 14 sesiones consecutivas diarias (3 h por sesión).
These drug doses (dissolved in sterile saline) were selected on the basis of previous studies (Caprioli et al. 2007a, b; 2008; 2009; Celentano et al. 2009). For half of the rats, the starting drug was heroin and for the other half it was cocaine, and each drug was paired with one of the two levers in a between-subject counterbalanced manner. At the beginning of each session, the appropriate lever was extended and the relative cue light was switched on. Completion of the task on the lever resulted in the delivery of the infusion (40 μl) over a 3-s period and in the retraction of the lever and the switching off of the cue light for a 40-s timeout period. The rats that did not spontaneously self-administer at least one infusion within the first 5 min of the session were placed with their forepaws on the lever to prime an infusion. This was repeated at times 60 and 120 min for rats that did not self-administer at least one infusion in time periods 5–60 and 60–120 min. These priming infusions were not included in data analysis. The schedule requirement to obtain an infusion was progressively increased from fixed ratio 1 (FR1) to FR5 according to the following schedule: FR1 on sessions 1–6, FR2 on sessions 7–8, and FR5 on sessions 9–14. The lever alternation continued on sessions 15–16, but upon completion of the task (FR5), the rats received a saline injection.

### Ultrasonic vocalizations

Ultrasonic vocalizations were recorded at baseline condition (3 min in a clean polycarbonate cage), during the period 0–30 min of the first two and the last two SA sessions, and again during the two sessions of saline SA (see Fig. 1). Avisoft UltraSoundGate condenser microphones capsule CM16 and Avisoft Recorder software (Version 3.2) were used. The recording settings included sampling rate at 250-kHz, 16-bit format. The recordings were processed using Avisoft SASLab Pro (Version 4.40) and a fast Fourier transformation (FFT). Spectrograms were generated with an FFT length of 1024 points and a time window overlap of 75% (100% Frame, Hamming window). The spectrogram was produced at a frequency resolution of 488 Hz and a time resolution of 1 ms. A lower cutoff frequency of 15 kHz was used to reduce background noise.

| Pair of sessions | 1-2 | 3-4 | 5-6 | 7-8 | 9-10 | 11-12 | 13-14 | 15-16 |
|------------------|-----|-----|-----|-----|------|-------|-------|-------|
| FR               | FR1 | FR2 | FR3 |
| Infusions        | Cocaine/Heroin | Saline |
| USVs             | ![microphone](image1.png) | ![microphone](image2.png) |

Fig. 1 Outline of the experiment. The microphones indicate the sessions during which USVs were recorded. Please note that during saline self-administration, the alternation between cocaine- and heroin-paired cues and lever position was maintained.

![Fig. 2](image3.png)

Fig. 2 Representative spectrograms for three main categories of 50-kHz USVs. a Frequency-modulated calls are defined as vocalizations continuously or discretely modulated, with a mean slope >0.2 kHz/ms or with one or more pitch-jumps in them, which is an instantaneous change in frequency. b Fixed frequency calls have no modulation, with a mean slope of less than 0.2 kHz/ms. c Trills vocalizations are characterized by a rapid, massive frequency excursion, either alone or in combination with other calls.

Distinct calls were identified on the basis of USV-free intervals ≥50 ms. Each call was visually and acoustically identified by a trained observer and assigned to 1 of 15 categories (Wright et al. 2010), which were then further classified into three main categories, based on previous literature (Brudzynski 2015): (1) “frequency-modulated” (FM) calls, characterized by a continuous or discrete frequency modulation (≥0.2 kHz/s), in either one or two or more directions; (2) “fixed frequency” calls, which were substantially flat USVs (mean change in frequency ≤0.2 kHz/s; (3) “trills” defined as rapid, massive frequency oscillations (including their combinations with vocalizations from other categories); and (4) 22-kHz calls. Representative spectrograms for these USV categories are reported in Fig. 2.

### Statistics

Self-administration data were analyzed with a three-way mixed ANOVA with repeated measures on the factor drug.
(cocaine vs. heroin) and the factor session, and with setting as a between-subject factor. When the sphericity assumption was violated, Greenhouse–Geisser correction was adopted. Post hoc $t$ tests for paired (when confronting lever pressing behavior on pairs of session from the same group) or unpaired samples (when confronting lever pressing behavior on sessions for the same substance between groups) were used to assess differences between sessions. Ultrasonic vocalization data were analyzed, due to high individual variability and lack of normal distribution, using Wilcoxon signed-rank tests for each subcategory. A drug preference score was obtained by calculating the ratio of USV emitted in response to cocaine versus heroin for each animal, after logarithmic normalization: log$_{10}$[(USV$_{coc}$ + 1)/(USV$_{hero}$ + 1)]. Two-way mixed ANOVA was run on these data followed by post hoc one-tailed $t$ tests, as the direction of change was clearly predicted on the basis of the working hypothesis. Data from three rats (two Non-Residents, one Resident) during the first session were lost due to hardware malfunctioning. Analysis was conducted using IBM SPSS 21.0 statistical software.

Separate analyses were conducted on the 50-kHz calls emitted immediately before (10 s) and immediately after the first ten infusions for sessions 13–14 and 15–16. Given the design of our study, there was large between- and within-subject variability in the number of cocaine, heroin, and saline infusions, as well as in their temporal distribution. Therefore, these data were analyzed using descriptive statistics only (see Figs. 7 and 8), as they were not suitable to inferential statistics.

**Results**

**Self-administration**

As illustrated in Fig. 3, cocaine SA and heroin SA were affected in a different manner by the setting. A three-way mixed ANOVA for repeated measures indicated significant main effects of session [$F_{6,150} = 56.700; p < 0.001$] and drug [$F_{1,25} = 23.754; p < 0.001$], and drug × setting [$F_{1,25} = 5.818; p = 0.024$], session × drug [$F_{6,15} = 16.176; p < 0.001$], and drug × session × setting [$F_{6,150} = 4.290; p = 0.012$] interactions. Virtually identical results were obtained analyzing earned infusions, with significant main effects of session [$F_{6,150} = 14.328; p < 0.001$] and drug [$F_{1,25} = 17.347; p < 0.001$], and drug × setting [$F_{1,25} = 5.230; p = 0.031$], and session × drug [$F_{6,15} = 6.786; p < 0.001$] interactions. No group differences were found for the saline SA sessions. The bottom panel of Fig. 2 illustrates the ratio of cocaine to heroin infusions. Two-way mixed ANOVA shown a main effect of setting ($F_{1,25} = 10.294; p = 0.004$).

**Ultrasonic vocalizations**

Figures 4 and 5 illustrate the number of 50-kHz USVs emitted during the first 30 min of drug SA for sessions 1–2 and 13–14. Overall, Non-Resident rats produced more USVs than Resident rats. However, Non-Resident rats emitted more USVs in response to cocaine than in response to heroin, especially during sessions 1–2 (Fig. 4), when they produced about twice as many USVs for cocaine as for heroin ($p = 0.039; r = 0.42$). In contrast, Resident rats emitted more USVs in response to heroin than to cocaine, especially during sessions 13–14 (Fig. 5), when they produced about three times as many USVs for heroin as for cocaine ($p = 0.044; r = 0.39$). Figures 4 and 5 also illustrate the log-normalized ratios of cocaine-induced over heroin-induced USVs, further indicating that Non-Resident rats vocalize more in response to cocaine than to heroin during the early SA sessions, whereas Resident rats vocalize more in response to heroin than to cocaine during the last SA sessions. Bottom panels in Figs. 4 and 5 show the drug preference score (calculated as described in the “Materials and methods” section) for Resident and Non-Resident rats. A two-way mixed ANOVA for repeated measures conducted on these data indicated a main effect of session ($F_{1,22} = 5.256; p = 0.032$) and of setting ($F_{2,44} = 4.006; p = 0.025$). Post hoc $t$ tests revealed a significant difference between Residents and Non-Residents at both early ($t_{54} = -1.732; p = 0.048$) and late training ($t_{54} = -1.790; p = 0.043$), but not for saline self-administration ($t_{54} = -0.597; p = 0.556$). Furthermore, the average of the scores of Non-Residents is significantly different from 0 for early training ($t_{113} = 2.081; p = 0.031$), whereas Resident rats’ scores differ from zero for late training ($t_{113} = 2.267; p = 0.022$).

The differential modulatory influence of setting on cocaine- versus heroin-induced USVs was critically dependant on the actual infusion of heroin or cocaine because it was not observable when the rats were exposed to the conditioned stimuli associated to drug infusion, as during saline SA on sessions 15–16 (Fig. 6). This phenomenon is even more evident when the calls emitted immediately before or after each infusion are considered. Figure 7 compares the frequency of preinfusion calls (10 s before infusion) for the first ten infusions of cocaine or heroin, on sessions 13–14, to that for the first ten infusions of saline, on sessions 15–16. Figure 8 illustrates a similar comparison for the calls emitted in the 40 s after each infusion. In the Resident group, the rats vocalized much more before and after heroin infusion than after saline infusion, whereas the call frequency for cocaine was similar to that for saline. In contrast, Non-Resident rats vocalized more before and after cocaine infusion than after saline infusion, whereas the call frequency for heroin was similar to that for saline.

There was no significant correlation between the number of heroin or cocaine infusions and the number of calls in any
session for either the Resident or the Non-Resident rats (all \( p \) values ≥ 0.2; data not shown).

Table 1 illustrates the number of 50-kHz USVs for each category. In Non-Resident rats, cocaine elicited more frequency-modulated calls relative to heroin during sessions 1–2 (\( p = 0.05 \), \( r = 0.40 \)). In contrast, Resident rats emitted more frequency-modulated (\( p = 0.032 \), \( r = 0.42 \)) and trills (\( p = 0.043 \), \( r = 0.39 \)) USVs in response to heroin relative to cocaine during sessions 13–14.

The rats emitted very few 22-kHz calls (about 1% of all recorded calls). The majority of these 22-kHz calls (181 out of 200) were emitted by a single Non-Resident rat on the first session of heroin SA.

Discussion

We report here three main findings. First, we found that the positive affective state (as indicated by 50-kHz USVs)
induced by cocaine versus heroin SA is modulated in a substance-specific manner by the setting of drug taking. On the basis of previous studies (Caprioli et al. 2007a, b; 2008; 2009), we hypothesized that heroin is more rewarding than cocaine when self-administered in a familiar home environment, whereas cocaine is more rewarding when self-administered outside the home. Overall, the findings reported here are in agreement with this hypothesis.

Second, in agreement with previous reports (Barker et al. 2010; Browning et al. 2011; Ma et al. 2010; Maier et al. 2012; Reno et al. 2013), we found that cocaine SA facilitates the emission of 50-kHz USVs and that this phenomenon is temporally related to drug infusion, as indicated by the fact that the call frequency was higher in the periods immediately before and after the infusions relative to the rest of the session. We have previously reported that rats tend to self-administer more cocaine when the setting of drug taking is distinct from the home environment (Non-Resident rats) relative to when the SA chamber is also the home environment (Resident rats) (Caprioli et al. 2007a). In contrast, Resident rats tend to self-administer more heroin than Non-Resident rats (Caprioli et al. 2008). We also conducted experiments in which rats were trained, as in the present study, to

Third, we report here for the first time that heroin increases 50-kHz USVs and that, as for cocaine, this effect is temporally linked to drug infusion. To the best our knowledge, no previous study has examined the emission of 50-kHz USVs in rats self-administering heroin, or even morphine (which, in any case, has a pharmacological profile distinct from that of heroin; e.g., Antonilli et al. 2005). We have previously reported that rats tend to self-administer more cocaine when the setting of drug taking is distinct from the home environment (Non-Resident rats) relative to when the SA chamber is also the home environment (Resident rats) (Caprioli et al. 2007a). In contrast, Resident rats tend to self-administer more heroin than Non-Resident rats (Caprioli et al. 2008). We also conducted experiments in which rats were trained, as in the present study, to
self-administer cocaine and heroin (at the same dosages used here) on alternate days (Caprioli et al. 2009; Celentano et al. 2009; Montanari et al. 2015). Under such conditions, Non-Resident rats took much more cocaine than Resident rats whereas the two groups self-administered more or less the same amount of heroin, suggesting that the two drugs affected the intake of one another. Virtually identical results were reported here (see Fig. 2). We have previously discussed in detail the possible reasons for the differential reinforcing effects of cocaine and heroin as a function of setting (Caprioli et al. 2007b; Badiani 2013; Badiani and Spagnolo 2013). For example, although the relationship between the reinforcing and the discriminative effects of addictive drugs is a controversial issue (e.g., Gossop 2001), it is interesting to notice that the setting can affect in opposite directions cocaine and heroin discrimination (Paolone et al. 2004; Caprioli et al. 2007b), much in the same way it affects the self-administration of these two drugs. Thus, it is possible that when a drug is more easily discriminated, it also becomes more easily reinforcing. Another possibility is that the differences in the reinforcing effects of cocaine and heroin as a function of setting depend on differences in the hedonic properties of the two drugs. Heroin might be more reinforcing at home than outside the home because it induces a more positive affective state in the former setting than in the latter, and vice versa for cocaine. To investigate this hypothesis, we used USVs as an index of the emotional state of the rat. Research done in the past 25 years has shown that rats use USVs to communicate their emotional state to other conspecifics (for a review, see Brudzynski 2015). In particular, it has been shown that rewarding stimuli, including drug of abuse, can enhance the emission of 50-kHz USVs (Mutschler et al. 2001; Barker et al. 2010; Maier et al. 2010; Browning et al. 2011; Mahler et al. 2013). Thus, it has been proposed that these USVs may be used as an index of positive affective states in the rat (Knutson et al. 2002).

In the present study, we found major effects of setting and drug SA on the emission of USVs. First of all, Non-Resident rats emitted about ten times more 50-kHz USVs than Resident rats during both drug SA and saline SA. The most likely explanation for this finding is the heightened state of arousal produced by the transfer to a novel test environment (see Maier et al. 2010). Second, the number of USVs greatly increased over sessions in both Resident and Non-Resident rats. Sensitization of USV emission after repeated exposure to addictive drugs has been reported previously (Mu et al. 2009). Third, and most important, we found that the rate of USVs emitted during drug SA was modulated in a substance-specific manner by the setting. Specifically, the ratio of cocaine-induced to heroin-induced USVs was greater in Non-Resident than in Resident rats. The modulatory influence of setting on the emission USVs during cocaine and heroin SA was dependent on the presence of these drugs because it was no longer observable when the rats were shifted to saline SA.

The results summarized above are consistent with a hypothesis discussed in detail in previous papers (Badiani 2013; Badiani and Spagnolo 2013). Briefly, it was proposed that a drug is perceived as less rewarding when its peripheral and central effects are at odds with the setting of drug taking, that is, when there is a mismatch between setting and drug effects. The sympathomimetic, arousing, and activating effects of cocaine (or amphetamine), for example, would be experienced as unsuitable to a safe, non-challenging, domestic environment. In contrast, the drowsiness and sedation produced by heroin would be experienced as unsuitable to an exciting, novel environment. A similar line of reasoning would apply not only to psychostimulants and opiates. We have shown that Non-Resident rats take much more ketamine (which, like cocaine, has activating and sympathomimetic effects; Hancock and Stamford 1999) than Resident rats (De...
Fig. 7 Pre-infusion calls. Rate of 50-kHz USVs (means ± SEM) in the 10 s before each of the ten first infusions on sessions 13–14 (heroin or cocaine infusions) versus sessions 15–16 (saline infusions). Due to great individual variability in number and timing of earned infusions, only descriptive statistics are displayed for this dataset (see “Materials and methods” section).

Fig. 8 Post-infusion calls. Rate of 50-kHz USVs (means ± SEM) during the 40 s after each of the ten first infusions on sessions 13–14 (heroin or cocaine infusions) versus sessions 15–16 (saline infusions). Due to great individual variability in number and timing of earned infusions, only descriptive statistics are displayed for this dataset (see “Materials and methods” section).
Luca and Badiani 2011), whereas Resident rats take more alcohol (which like heroin causes, at least initially, drowsiness and sedation; Morean and Corbin 2010) than Non-Resident rats (Testa et al. 2011).

The mismatch hypothesis would also account for an intriguing result of the present study, that is, for the fact that the modulatory effect of setting on the emission of USVs during drug SA changed in a substance-specific manner over time. Resident rats exhibited in fact no significant differences in the number of USVs emitted during heroin versus cocaine SA on sessions 1–2, whereas they emitted about three times more USVs during heroin SA relative to cocaine SA on sessions 13–14. It is possible that this was due to the repeated exposure to the testing procedures, including cue light presentation, lever extension, and drug infusion induced a certain degree of arousal, which waned with repeated testing. In contrast, Non-Resident rats emitted twice as many USVs during cocaine versus heroin SA on sessions 1–2, whereas there were no significant differences on sessions 13–14. It is possible that this was due to the repeated exposure of Non-Resident rats to the SA chamber, which might have blunted, but not erased, the relative novelty of the setting. However, it should be noted that when the analysis was limited to the USVs emitted immediately before or after drug infusion (Figs. 7 and 8), Non-Resident rats vocalized more before/after cocaine infusion than after saline infusion, whereas the number of peri-infusion calls for heroin was similar to that for saline.

While the mismatch hypothesis predicted greater rewarding effects of heroin in Resident versus Non-Resident rats and of cocaine in Non-Resident versus Resident rats, it did not necessarily predict greater aversive effects of heroin in Non-Resident versus Resident rats and of cocaine in Resident versus Non-Resident rats. In any case, under the testing conditions of the present study, the rats emitted very few 22-kHz USVs, which are thought to reflect aversive states (Blanchard et al. 1991, 1992; Calvino et al. 1996; Covington and Miczek 2003; Koo et al. 2004; Lee et al. 2001; Lore et al. 1976; Mutschler and Miczek 1998; Portavella et al. 1993; Thomas et al. 1983; Vivian and Miczek 1991). Interestingly, the majority of the very few 22-kHz calls recorded in our study (181 out of 200) were emitted by a single Non-Resident rat on the first session of heroin SA.

What are the neurobiological mechanisms responsible for the differential influence of settings on the emission of heroin- and cocaine-elicited calls? It has been previously shown that the intravenous administration of heroin and cocaine at doses identical to those used in present experiments differentially activate dorsal striatum neurons in Resident versus Non-Resident rats (Celentano et al. 2009). Given the role of the striatal complex in the production of 50-kHz USVs (Barker 2010), further studies are necessary to investigate whether this differential neuronal activation is at least in part responsible for the findings reported here.

In conclusion, the present study shows that the setting of drug administration modulates in a substance-specific manner not only the reinforcing and interoceptive effects of cocaine versus heroin (as shown in previous studies) but also the ability of these drugs to induce positive affective states, at least as reflected by 50-kHz USV. In particular, we have shown that a given setting of drug taking can modulate in opposite manner all aspects of heroin versus cocaine reward: intake (Caprioli et al. 2007a, 2008, 2009; Celentano et al. 2009), motivation

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**Table 1** Number (means ± SEM) of trills, frequency-modulated (FM) calls, and fixed frequency (FF) 50-kHz USVs (see text), emitted by Non-Resident and Resident rats during the first 30 min of drug SA (sessions 1–2 and 13–14) and saline SA (sessions 15–16)

| Session       | USV category | Residents | Non-Residents | All          |
|---------------|--------------|-----------|---------------|--------------|
|               |              | Cocaine lever | Heroin lever | Cocaine lever | Heroin lever | Cocaine lever | Heroin lever |
| 1–2 drug SA   | Trills       | 0.58 ± 0.49 | 1.33 ± 1.33  | 8.33 ± 3.70  | 2.92 ± 1.59  | 4.18 ± 1.78  | 2.125 ± 1.03 |
|               | FM           | 4.92 ± 2.22 | 3.33 ± 1.70  | * 45.17 ± 14.13 | 24.25 ± 13.71 | * 25.4 ± 7.66 | 13.79 ± 7.10 |
|               | FF           | 5.58 ± 1.87 | 8.67 ± 6.63  | 24.00 ± 5.93  | 16.00 ± 8.38  | 15.96 ± 3.90  | 12.33 ± 4.53 |
|               | Total        | 10.23 ± 3.91 | 13.33 ± 5.70 | * 78.36 ± 20.65 | 43.17 ± 23.57 | 45.55 ± 12.59 | 28.25 ± 12.26 |
| 13–14 drug SA | Trills       | 1.92 ± 0.81 | * 16.62 ± 9.00 | # 58.71 ± 29.70 | 68.00 ± 32.83 | 31.37 ± 16.12 | 43.26 ± 17.97 |
|               | FM           | 21.08 ± 10.28 | * # 53.31 ± 23.65 | # 299.29 ± 186.02 | # # 182.71 ± 48.55 # 165.33 ± 98.68 | # # 120.41 ± 29.94 |
|               | FF           | 14.69 ± 6.13 | 28.31 ± 13.76 | 100.86 ± 42.73 | 66.07 ± 14.71 | 59.37 ± 23.52 | 47.89 ± 10.58 |
|               | Total        | 37.69 ± 16.94 | * # 98.23 ± 45.61 | # 458.86 ± 255.88 | # 316.79 ± 90.55 ## 256.07 ± 136.90 # # 215.55 ± 55.20 |
| 15–16 saline SA | Trills     | 1.38 ± 0.94 | 3.23 ± 2.60  | 14.43 ± 4.92  | 42.43 ± 20.02  | 8.15 ± 2.84  | 23.55 ± 10.96 |
|               | FM           | 7.92 ± 2.97 | 6.77 ± 3.02  | 44.14 ± 7.58  | 69.64 ± 28.73  | 26.70 ± 5.43  | 39.37 ± 15.94 |
|               | FF           | 9.62 ± 4.37 | 13.85 ± 5.09  | 38.57 ± 7.32  | 58.29 ± 28.35  | 24.63 ± 5.12  | 36.89 ± 15.27 |
|               | Total        | 18.92 ± 7.99 | 23.85 ± 8.16  | 97.14 ± 15.78  | 170.36 ± 75.91 | 59.48 ± 11.73 | 99.81 ± 41.41 |

*Significant differences (p ≤ 0.05) between heroin- and cocaine-induced calls; # , ## significantly more (p ≤ 0.05 and p ≤ 0.01, respectively) drug-induced calls on sessions 13–14 relative to the corresponding saline session (sessions 13–14)
(progressive ratio procedures; Caprioli et al. 2007a, 2008, 2009; Celentano et al. 2009), choice (Caprioli et al. 2009), drug discrimination (Paolone et al. 2004; Caprioli et al. 2007b), and affect (present study). It is important to notice that the setting does not influence all drug effects in the same way. We have previously shown that repeated administrations of heroin or morphine produce greater psychomotor sensitization in Non-Resident than in Resident rats (Badiani et al. 2000; Paolone et al. 2003, 2007), as previously reported for amphetamine and cocaine (Badiani et al. 1995a, b; Crombag et al. 1996; Browman et al. 1998). That is, psychomotor sensitization and rewarding effects can be modulated in opposite directions by the setting, and this opposite modulation has been observed to occur in parallel (e.g., Caprioli et al. 2008). Furthermore, some effects of drugs do not appear to be susceptible to the manipulation of setting investigated here. Tolerance to the analgesic effect of morphine, for example, develops in exactly the same way in Resident and Non-Resident rats (Paolone et al. 2003).

The effects of setting on cocaine versus heroin reward may explain the findings of studies conducted in human addicts, showing distinct setting preferences for cocaine versus heroin use (Caprioli et al. 2009; Badiani and Spagnolo 2013), and in rat models of drug relapse, showing differential vulnerability to cocaine versus primed reinstatement of drug seeking after a period of ext (Montanari et al. 2015). Taken together, these findings indicate the importance of taking into account the substance-specific aspects of drug use and misuse (Badiani et al. 2011; Badiani 2013).

Acknowledgments This work was supported by grants from Sapienza University of Rome (C26A12L24N) and from the University of Sussex (SDF-SA027-05). Husbandry and procedures were in accordance with the Italian Law on Animal Research (DLGS 116/92) and with the guidelines for the care and use of laboratory animals issued by Italian Ministry of Health, the country in which the experiments were performed.

Compliance with ethical standards

Conflict of interest The authors report no biomedical financial interests or potential conflicts of interest.

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