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Interaction of the phencyclidine model of schizophrenia and nicotine on total and categorized ultrasonic vocalizations in rats

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Abstract

Patients with schizophrenia smoke cigarettes at a higher rate than the general population. We hypothesized that a factor in this comorbidity is sensitivity to the reinforcing and reinforcement-enhancement effects of nicotine.

Phencyclidine (PCP) was used to model behavioral changes resembling negative symptoms of schizophrenia in rats. USVs in rats have been used to measure emotional states, with 50 kHz USVs indicating positive states and 22 kHz indicating negative. Total and categorized numbers of 22 and 50 kHz ultrasonic vocalizations (USVs) and USVs during a visual stimulus (e.g. a potential measure of reinforcement-enhancement) were examined in rats following .injection ofh PCP (2.0 mg/kg), and/or nicotine (0.2 or 0.4 mg/kg) daily for 7 days. PCP was then discontinued and all rats received nicotine (0.2 mg/kg and 0.4 mg/kg) and PCP (2.0 mg/kg) on 3 challenge days.

PCP acutely decreased 50 kHz vocalizations while repeated nicotine potentiated rates of vocalizations, with similar patterns during light presentations. Rats in the PCP and nicotine combination groups made more 50 kHz vocalizations compared to control groups on challenge days.

We conclude that PCP may produce a reward deficit that is shown by decreased 50 kHz USVs, and behaviors post-PCP exposure may best model the comorbidity between schizophrenia and nicotine.

Keywords

ultrasonic vocalization; nicotine; phencyclidine; schizophrenia

Introduction

Tobacco use is a serious health problem with approximately 400,000 people dying annually in the United States from nicotine-related concerns (Center for Disease Control, 2014). Tobacco addiction is an especially severe problem within the population of schizophrenic patients; between 50–90% of the schizophrenic population use tobacco in some form, compared to 20–35% of the general population of the United States (Kumari & Postma,

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2005). This increased dependence on tobacco products leads to damaging health problems in this subpopulation, shown by increased incidence of cardiovascular and respiratory diseases as well as a smoking-related mortality rate that is 2–3 times higher than the general public (Buda et al., 1988; Hennekens et al., 2005; Mortensen & Juel, 1990; Saha et al., 2007).

While a number of hypotheses have been proposed as to the factors involved in the comorbidity of tobacco use and schizophrenia, there is currently no consensus in the field. A number of studies suggest a role of reward in the comorbidity between tobacco use and schizophrenia. For example, anhedonia, which is defined as the lack of an ability to gain pleasure or reward, is a common symptom seen in many schizophrenics (Gopalaswamy & Morgan, 1986). Patients with schizophrenia rank smoking above other natural rewards, more so than non-mentally ill subjects (Smith et al., 2002). These findings implicate an alteration of reward-related behavior in patients with schizophrenia who smoke, yet there is a patent lack of preclinical research on the reward system and reinforcement efficacy in this comorbidity.

This study aimed to determine how reward and affective states are affected by nicotine and phencyclidine (PCP), which was used to model certain symptoms of schizophrenia in rats. Ultrasonic vocalizations have recently come to attention as potential measures of the emotional and motivational states of rats, including reward (Panksepp & Burgdorf, 2000; Browning et al., 2011; Maier et al., 2012; Mu et al., 2009). Specifically, 50 kHz vocalizations are typically emitted in reinforcer-associated contexts (Brunelli et al., 2006; Knutson et al., 1998; Panksepp & Burgdorf, 2000; Sales, 1972; Schwarting et al., 2007; White et al., 1990) while 22 kHz vocalizations are generally associated with aversive situations (Francis, 1977; Jelen et al., 2003; Tonoue et al., 1986; Wohr et al., 2005; Mead et al., 2008; Sun et al., 2010). Wright (2010) further categorized vocalizations into subcategories that may lead to better classification of emotional and motivational states. To date, the few studies examining PCP have shown that PCP decreased tickling-induced 50 kHz vocalizations and increased 22 kHz vocalizations (Boulay et al., 2013; Tunstall et al., 2009), behavior suggestive of a more negative affective state after PCP exposure, which may be considered a model of anhedonia seen in schizophrenics.

While there have been relatively few studies on the impact of an animal model of schizophrenia on USVs, there have been even fewer on nicotine and none on the interaction of effects of phencyclidine and nicotine. Data so far suggest that repeated nicotine potentiated USVs while acute administration produced no effect (Simola et al., 2012, 2014). However, these studies only examined the general reinforcing and potentially aversive effects of nicotine. Nicotine has been suggested to have dual reinforcement properties, with a relatively weak primary reinforcing effect and a reinforcement-enhancing property, both of which contribute to tobacco addiction (Caggiula et al., 2009). The reinforcement-enhancement effect is typically defined as enhancement by nicotine of the reinforcing effects of other non-drug stimuli (Liu et al., 2007) and has been reliably demonstrated in preclinical models (Barrett & Odum, 2011; Raiff & Dallery, 2006, 2008, 2009; Thiel et al., 2009). While the dual-reinforcement model has been examined in healthy patients, there is no research comparing these two properties of nicotine in a preclinical model of schizophrenia symptoms and none examining these properties utilizing ultrasonic vocalizations.

The purpose of this study was threefold. First, PCP produces differential behavioral responses after acute and chronic exposure as well as after discontinuation (Jentsch & Roth, 1999; Mouri et al., 2007; Nabeshima et al., 1983) and these three frequently used treatment regimens have not been compared using the ultrasonic vocalization paradigm to evaluate its effect on positive and negative affective states. By evaluating the effects of acute and chronic treatment in addition to post-cessation of PCP treatment, this study aimed to illuminate which treatment regimen may be most sensitive to the effects of nicotine. Second, as discussed, this study aimed to show how nicotine interacts with the PCP treatment regimens as well as to detail the acute and chronic effects of nicotine in the USV paradigm. Finally, this study proposed a putative model of comparing the primary reinforcing and reinforcement-enhancement effects of nicotine by examining ultrasonic vocalizations before and during a brief visual stimulus. This visual stimulus (VS) is mildly reinforcing to a rat (Berlyne & Koenig, 1965; Glow & Russell, 1975) and is frequently used in testing the reinforcement-enhancing effect (Barrett & Bevins, 2012; Chaudhri et al., 2007; Donny et al., 2003; Palmatier et al., 2006). Based on these findings, reinforcement-enhancement was hypothesized to be seen as an increase in ultrasonic vocalizations during a light presentation. Overall, examining the individual effects of nicotine and phencyclidine, the interactions between these compounds and how a visual stimulus may affect ultrasonic vocalizations after drug treatment could preclinically delineate factors involved in the schizophrenia and nicotine addiction comorbidity.

Methods

Subjects

Forty-six adult male Sprague Dawley rats (275–299g upon arrival, Harlan, Indianapolis, IN, USA) were used in the final analysis. Two animals were excluded due to extremely high levels of 22 kHz vocalizations during an early session (e.g. over 500 vocalizations in 5 min), suggesting an acute stressor or pain that was not present in other animals. They were individually housed in clear rectangular polycarbonate tubs (48.3 cm × 26.7 cm × 20.3 cm) under 12-h light/dark conditions to avoid potential isolation-induced vocalizations. Experiments were conducted during the light cycle (light on between 06:00 and 18:00h). Room temperature was maintained at 22±1°C with a relative humidity of 45–60%. Water was continuously available and food was freely available. Animals were allowed 5 days of habituation to the animal facility before being used in experiments during which they were handled for 5 min per rat by each experimenter. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

Apparatus

Eight identical two-way shuttle boxes custom designed and manufactured by Med Associates (St. Albans, VT) were used. Each box was housed in a ventilated, sound-insulated isolation cubicle (96.52 cm W×35.56 cm D ×63.5 cm H). Each box was 64 cm long, 30 cm high (from grid floor) and 24 cm wide, and divided into two equal-sized compartments by a white PVC partition with an arch style doorway (15cm high × 9cm wide at base). An aluminum hurdle (4cm high) was placed between the two compartments. The grid floor consisted of 40 stainless steel rods with a diameter of 0.48 cm, spaced 1.6 cm

apart center to center. Two cue lights (28 volts) were mounted at the top of each compartment and a houselight (50 volts) was located at the top of each soundproof chamber. All testing procedures were controlled by Med Associates programs running on a computer. Background noise (approximately 74dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle.

In each two-compartment chamber, an ultrasonic vocalization microphone (P48 Avisoft Bioacoustics / Emkay Microphone, Avisoft Bioacoustics, Berlin, Germany) was mounted on the ceiling of the. The microphone was connected to a computer via an E-MU 0404 USB Audio device. Acoustic data were displayed in real time by the Avisoft RECORDER, a multi-channel triggering hard-disk recording software (version 3.4; Avisoft Bioacoustics), and were recorded at a sampling rate of 192 kHz in 16 bit format (version 4.51; Avisoft Bioacoustics). Recording of vocalization was started exactly 5 s prior to the Med Associates program and was manually synchronized before analysis.

Spectrograms were generated from the sound files using RavenPro 1.5 (Cornell Laboratory of Ornithology, Ithaca New York: 512 sample Fourier transformation with a Hann window function; filter bandwidth 135 Hz; frequency resolution 93.8 Hz; grid time resolution 2.13 ms). Two trained scorers hand-counted both the 50 kHz and 22 kHz vocalizations with the criteria that 22 kHz vocalizations occurred in the range between 20 and 25 kHz and 50 kHz occurred between 30 and 95 kHz. Scorers classified the 50 kHz vocalizations into 14 different categories (complex, composite, downward ramp, upward ramp, flat-trill combination, flat, short, inverted u, multistep, trill, trill with jumps, step down, step up, and split) based on the parameters set forth in Wright and colleagues (2010). The inter-rater reliability was assessed using a subset of 700 vocalizations, with 94% of calls receiving the same classification between scorers.

Procedure

Each rat was habituated to the chambers for 30 min per day for 2 days. During these habituation sessions, rats were placed into the chambers with a house light on. Rats were pre-injected with nicotine in their home cage 2 h after each habituation session, in order to potentially attenuate an initial aversion to nicotine (Bevins et al., 2001). After habituation, rats were randomly assigned to 6 groups based on the drugs administered before each drug testing session: SAL-SAL, SAL-NIC 0.2 mg/kg, SAL-NIC 0.4 mg/kg, PCP-SAL, PCP-NIC 0.2 mg/kg, and PCP-NIC 0.4 mg/kg.

For a total of 7 daily drug testing sessions, rats were injected with drug (SAL, 2.0 mg/kg PCP and the corresponding NIC dose) and then placed into the chambers for 30 min. Ultrasonic vocalization was recorded during the entire 30-min session. During this time, lights were presented on a variable time (VT) 95 s schedule with an average of 19 presentations per session. Light presentations involved the brief onset of the cue lights (5 s) and termination of the house light for 5 s. The exact time that the cue light was presented was recorded. Vocalizations were recorded over all 7 days of testing.

After the drug testing, rats underwent 7 days of no PCP or nicotine exposure. Rats were then placed into the vocalization recording chambers for a series of challenge days. During these

challenge days, all rats, regardless of their past drug treatment history, were tested under the same drug condition. On the 1st challenge day they received NIC (0.2 mg/kg). This was followed by NIC (0.4 mg/kg) and PCP (2.0 mg/kg) challenge days. The challenge days were presented in this order so as to keep constant the number of days of PCP non-exposure across animals.

Drugs

(−)Nicotine tartrate salt (Sigma, St. Louis, MO) was dissolved in 0.9% saline and adjusted to a pH of 7.0 ± 0.2 with a dilute NaOH solution. The high dose (0.4 mg/kg) was chosen based on previous research showing reinforcement-enhancement at that dose (Palmatier et al., 2007, 2009) and, due to the potential of a ceiling effect, a further lower dose (0.2 mg/kg) was chosen on the basis of pilot data. Phencyclidine hydrochloride (PCP, a gift from NIDA Chemical Synthesis and Drug Supply Program) was obtained by mixing drug with 0.9% saline. Saline and PCP (2.0 mg/kg) were administered s.c. 10 min prior to placement in the chamber and nicotine (0.2 or 0.4 mg/kg) was administered s.c. 5 min prior to chamber placement. All doses are expressed as base. The dose of PCP was chosen on the bais of unpublished pilot data suggesting that this dose, combined with the nicotine doses, would affect ultrasonic vocalizations). This dose has also been used to model symptoms of schizophrenia in a similar regimen (Abdul-Monim et al. 2003, 2007; Jentsch et al. 1997).

Statistical Analyses

In order to determine if the vocalizations differed between the six groups and seven testing sessions, multiple ANOVAs were run. Factorial two-way ANOVAs targeted at the hypotheses were run to examine 50 kHz and 22 kHz vocalizations. In addition, two one-way ANOVAs were used to determine differences in the 50 kHz category types independently for the testing sessions, with group as the independent variable and the number of vocalizations in each category as the dependent variable. One-way ANOVAs were run to examine the differences on the challenge days with group as the independent variable and the number of 50 kHz vocalizations as the dependent variable. Fisher's least significant difference tests (LSD) were used as post hoc tests to attempt to protect from false positives with the minimum mean difference (mmd) reported. The significance level was set at 5% and was two-tailed.

Results

Total 50 kHz

Using targeted one-way ANOVAs to examine the hypotheses, we first looked at how PCP would affect vocalizations over days (see Figure 1). On Day 1, we found a marginally significant difference between PCP-SAL and SAL-SAL groups [$F(1,14)=4.509$, $p=0.053$]. PCP did not change 50 kHz vocalizations on days 2 through 7 (ps≥ 0.1). Fisher's LSD tests showed that the PCP-SAL group had lower vocalizations on the first day compared to saline ($p<0.02$).

A one-way ANOVA comparing nicotine groups to saline was run to determine the effects of nicotine on 50 kHz vocalizations (see Figure 2). There was a significant difference between

groups on Day 7 [F(2,23)=4.99, $p<0.05$) but no significant differences on days 1 through 6 ($ps\geq0.2$). Fisher's LSD tests showed that the low dose nicotine group was significantly different from saline ($p<0.01$). These results suggest that, after repeated exposure, low dose nicotine led to increased vocalizations in comparison to saline.

Finally, ANOVAs were run to compare the interaction between PCP and nicotine. There were no significant differences between the SAL-NIC 0.2 and PCP-NIC 0.2 groups on any days ($ps\geq0.3$). There were also no significant differences between the SAL-NIC 0.4 and PCP-NIC 0.4 groups ($ps\geq0.2$). These results suggest that the combination of nicotine and PCP did not affect vocalization rates after acute and repeated treatment.

50 kHz during Light

Examining 50 kHz USVs during light presentations, we found similar results (see Figure 3). There was a significant difference on Day 1 [F(1,14)=5.02, $p<0.05$] and a marginally significant difference on Day 2 [F(1, 14)=4.23, $p=0.06$]. There were no significant differences on days 3 through 7 ($ps\geq0.4$). Fisher's LSD tests showed that the PCP-SAL groups had lower 50 kHz USVs on Days 1 and 2 ($ps\leq0.02$). These results suggest that PCP decreased 50 kHz vocalizations during light on the first day and marginally decreased vocalizations on Day 2.

In comparing 50 kHz USVs emitted during the light presentations for nicotine, we again found that there was a significant main effect of group [see Figure 4, F(2,23)=5.35, $p<0.05$]. LSD tests showed that there was a significant difference between both the low dose group ($p<0.05$) and the high dose group ($p<0.05$) compared to saline. There were no significant differences between groups on Days 1 through 6 ($ps\geq0.2$). These results show that both low and high dose nicotine groups had higher levels of 50 kHz USVs during lights on the final day of testing.

Finally, interactions between nicotine and PCP were tested. No significant differences on any days were found when comparing SAL-NIC 0.2 to PCP-NIC 0.2 ($ps\geq0.09$) or SAL-NIC 0.4 to PCP-NIC0.4 ($ps\geq0.2$). These results suggest that the combination of nicotine and PCP did not affect vocalization rates after acute and repeated treatment during the light stimulus.

50 kHz Categories

In addition to total vocalizations, the 50kHz USVs were separated into 13 different categories. Separate one-way ANOVAs were run to look at differences in the types of 50kHz calls emitted on the seven days of treatment (see Figure 5). On the first day, the groups showed significant differences in composite vocalizations [F(5,39)=2.87, $p<0.05$], as well as multistep vocalizations [F(5,39)=3.75, $p<0.01$]. Pairwise comparisons (LSDmmd=19.98) showed that the SAL-SAL group had more composite vocalizations than all other groups ($ps\geq0.014$) and more multistep calls than all other groups (LSDmmd=18.62; $ps\geq0.014$). In addition, there was a trend for a difference in upward ramp vocalizations between the groups [F(5,39)=2.45, $p=0.05$]. Pairwise comparisons (LSDmmd=43.72) revealed that the SAL-SAL group produced more upward ramp vocalizations than the SAL-NIC 0.4, PCP-NIC 0.2 and the PCP-NIC 0.4 groups (see Figure 5, $ps\geq0.019$).

There were no significant differences in the types of calls given by the groups on days 2 through 6 ($p \geq 0.08$). On day seven there was a significant difference in the multistep calls between the six groups [$F(5,39)=2.67$, $p<0.05$]. Pairwise comparisons (LSDmmd=7.73) showed that the PCP-NIC 0.4 group had significantly fewer multistep vocalizations than the SAL-SAL and SAL-NIC 0.4 groups ($p \leq 0.05$). In addition, on day three, there was a significant difference in the number of uncategorized calls between the six groups [$F(5,39)=2.71$, $p<0.05$]. Pairwise comparisons (LSDmmd= 6.17) showed that the SAL-NIC 0.4 group had significantly more uncategorized calls than all of the other treatment groups ($p \leq 0.05$). These calls looked similar to 50 kHz vocalizations but appeared in the 22 kHz range (see Figure 6).

22 kHz Vocalizations

There were no significant effects on any of the measures on total 22 kHz USVs (ps≥0.3) or 22 kHz USVs during light stimuli (ps≥0.2). Only 19 total 22 kHz USVs were made on all drug testing days and only 1 vocalization was emitted during the light stimuli over days.

Challenge Days

The three challenge days showed significant differences in number of 50 kHz calls. On challenge day one, when rats were administered a low dose of nicotine (0.2 mg/kg), there was a significant difference in number of calls between the six groups, [see Figure 7, $F(5,33)= 6.01$, $p< 0.001$]. Pairwise comparisons (LSDmmd=352.19) indicated that the SAL-NIC 0.4 group emitted significantly more vocalizations than all other groups ($p \leq 0.005$) except the SAL-NIC 0.2 group ($p=0.051$). In addition, the SAL-NIC 0.2 group produced significantly more vocalizations than the SAL-SAL group ($p=0.01$).

On challenge day two, when rats were administered a high dose of nicotine (0.4 mg/kg), there was a significant difference in the number of calls between the six groups [see Figure 4, $F(5,33)=3.25$, $p<0.05$]. Pairwise comparisons (LSDmmd=229.16) indicated that the SAL-NIC 0.4 group made significantly more vocalizations than all other groups ($p<0.05$).

On challenge day three, when rats were administered PCP, there was a significant difference in the number of calls between the six groups [see Figure 5, $F(5,33)=8.35$, $p<0.02$]. Pairwise comparisons (LSDmmd=503.99) showed that the PCP-NIC 0.2 and PCP-NIC 0.4 groups were not significantly different ($p=0.06$), and these two groups produced significantly more vocalizations than the other groups ($p<0.001$).

Discussion

First, we found that nicotine and PCP altered total vocalizations in an opposing fashion: PCP, after acute treatment, seemingly decreased total 50 kHz vocalizations while nicotine treatment led to higher total vocalizations compared to control groups but only after repeated exposures. Similar findings were seen in the 50 kHz vocalizations during the visual stimulus presentations. There were no differences in 22 kHz vocalizations. When considering categories of vocalizations, specific subtypes were differentially affected by nicotine and PCP treatment. Finally, no consistent interactions were seen during acute and chronic PCP

treatment but only animals that had received both nicotine and PCP had higher vocalizations in compared to all other groups on challenge sessions.

Examining overall vocalizations elicited throughout the session, acute PCP exposure appeared to produce a more negative affective state, as shown by a decrease in 50 kHz vocalizations. This finding is analogous to the recent study showing that PCP attenuated tickling-induced 50 kHz vocalizations (Boulay et al., 2013). While this experiment had a number of methodological differences (i.e. different age of rat, tickling-induced vocalizations, different dosage), the similarities between the findings suggest that the attenuation of 50 kHz USVs by PCP is a relatively robust effect. Boulay and colleagues (2013) suggested that this decrease may model anhedonia (a negative symptom of schizophrenia) and the results of the present study support this idea. Contrary to research on "aversive" vocalizations (Tunstall et al., 2009), PCP did not increase 22 kHz vocalizations in this paradigm, but only altered 50 kHz USVs, suggesting that decreases in the 50 kHz vocalizations might be a better measure to examine reinforcement differences.

Contrary to the effects of PCP on 50 kHz vocalizations, the group receiving the high dose of nicotine had higher levels of vocalizations associated with positive affective states in comparison to control groups, but this effect was only seen after repeated nicotine exposure. In previous studies, acute nicotine did not produce significant changes in total numbers of 50 kHz USVs (Simola et al., 2012) but repeated nicotine treatment led to increased vocalizations over days (Simola et al. 2013). Our findings support these previous findings in terms of acute and chronic effects, with nicotine producing no significant effect on the first day but causing increased numbers of USVs in comparison to saline on the final day of treatment. Similarities between these results suggest that repeated exposure may be necessary to see the reinforcing activity of nicotine in the ultrasonic vocalization paradigm. Repeated nicotine exposure is integral in other paradigms and appears to be integral to development of reinforcement-enhancement effects of nicotine (Barrett & Odum, 2011; Palmatier et al., 2007; Raiff & Dallery, 2006) As alterations in rates of ultrasonic vocalizations may potentially be used to elucidate mechanisms behind drug dependence, such as sensitization and tolerance (Taracha et al., 2007), exploring the mechanisms behind this repeated effect of nicotine and its relationship to behavioral and neurobiological factors involved in drug dependence is vital to further detail the importance and validity of examining ultrasonic vocalizations for drug addiction measures.

Vocalizations during light presentations were examined, in addition to overall vocalizations, to determine if USVs might be sensitive enough to illuminate differences in the reinforcement-enhancement effects of drugs. Reinforcement-enhancement was hypothesized to be seen as an increase in ultrasonic vocalizations during a light presentation. However, overall vocalizations did not seem to change significantly during light presentations (not shown) and temporal patterns were fundamentally similar to those seen during no light presentation, although the effects of PCP lasted longer when examining vocalizations during light. It is possible that this paradigm is not sensitive enough to fully detail the reinforcement-enhancement effect of drugs. It is also possible that the reinforcing value of the stimuli might not be sufficient to detect differences. The non-contingent nature of the stimuli could potentially reduce the reinforcing value of the visual stimulus or the length of

the visual stimulus may have been too short, as others have used significantly longer stimuli (Barrett & Bevins, 2012; Caggiula et al., 2002; Chaudhri et al., 2007; Donny et al., 2003; Palmatier et al., 2006). Future research to determine if the reinforcement-enhancement effects of nicotine can be seen in this paradigm is warranted, as ultrasonic vocalizations do not rely on locomotor activity, which can significantly affect operant behavior typically used to study reinforcement-enhancement.

We also found that different subtypes of 50 kHz vocalizations were differentially affected by acute treatment of both PCP and nicotine. Both PCP and nicotine significantly decreased composite and multistep subtypes of vocalizations after acute treatment. Rats that had previously received nicotine had fewer upward ramp vocalizations yet the numbers of total vocalizations were not significantly different. All of the subtypes affected by both nicotine and phencyclidine exposure were frequency-modulated; there were no differences in either total or light-paired flat vocalizations. In addition, nicotine did not alter trill vocalizations, in agreement with the findings of Simola and colleagues (2014). The importance of subtypes is not currently known but different categories of vocalizations have been hypothesized to have different behavioral significance (Wright et al., 2010). Since 50 kHz calls can occur in potentially aversive situations (see Wohr et al., 2008 for discussion), examination of specific subcategories of 50 kHz vocalizations might be better suited to examining reward and reinforcement. For example, frequency modulated calls such as trills are associated with appetitive stimuli (Ahrens et al., 2009; Burgdorf et al., 2007; Burgdorf & Panksepp, 2006; Wohr et al., 2008), while flat vocalizations are observed in non-appetitive situations such as during short periods of isolation (Stevenson et al., 2009; Wohr et al., 2008).

Vocalization analysis not only uncovered differences in established 50 kHz subtypes but additional vocalizations resembling 50 kHz subtypes were seen in the range associated with 22 kHz vocalizations (see Figure 7). These vocalizations were placed into the uncategorized vocalization subtype and a potentiation of total vocalizations in this category was seen in the SAL-NIC 0.4 group. Given that differences in 50 kHz USVs may have different behavioral implications, it is possible that subtypes of 22 kHz vocalizations might also be associated with different features. 22 kHz calls have been examined for the temporal nature (Brudzynski et al. 1993) and frequency of modulation (see Litvin et al., 2006, Vivian & Miczek, 1993a, b) and considerably short 22 kHz vocalizations have been found during drug treatment (Barker et al. 2010). To the authors' knowledge, USVs in the 22 kHz range resembling the vocalizations found in this experiment have not previously been identified. Further replication of this USV subtype and experimentation involving devocalization surgery is necessary but these results are intriguing, especially given the differences seen in these vocalizations after nicotine treatment.

The previous discussion has focused on the acute and chronic exposure to PCP and nicotine. In these two treatment regimens, no interaction was found between nicotine and phencyclidine. Interactions were, however, seen on challenge days. On the nicotine challenge days, only a basic sensitization effect was seen; previous exposure to nicotine (either with or without PCP on board) led to increased 50 kHz vocalizations. However, the most interesting findings occurred on the PCP challenge day. Rats that had received both nicotine and PCP had higher levels of vocalizations on the PCP challenge day; this was

significantly different from rats that had only received PCP and groups that had only received nicotine.

The finding that an interaction between PCP and nicotine is only seen on challenge days is consistent with findings on the differential effects of PCP treatment regimens on schizophrenia-related behaviors. Acute exposure, chronic exposure and cessation from PCP treatment consistently show behavioral differences in a number of paradigms, specifically in relation to negative symptoms (for review, see Neill et al., 2013). Here we see that the interaction between the two drugs is only seen after PCP treatment has been discontinued. It is possible that rodents in this animal model of negative symptoms of schizophrenia might be more sensitive to reinforcing effects of nicotine after cessation of PCP treatment, given the increase in 50 kHz USVs seen only in the groups with the pairing. Thus, this discontinuation model may be better suited to modeling negative symptoms of schizophrenia (Ellenbroek & Cools, 2000). It may be possible, therefore, that schizophrenia patients with a higher preponderance of negative symptoms may be more sensitive to the reinforcing effects of nicotine, which could increase nicotine use. While some human research supports this suggestion, such as work by Ahnallen & colleagues (2012) showing that the severity of anhedonia is correlated with the severity of nicotine dependence, it is still speculative and there is a clear need for more research on this idea. Another possibility is that effects on the PCP challenge day may be influenced by the conditioning between PCP and nicotine. The interoceptive state of PCP could act as a cue to “predict” nicotine, with the lack of nicotine or the previous conditioning of the PCP/nicotine combination influencing USVs. Further research on the effects of conditioning is needed to elucidate the mechanisms behind the challenge day difference.

Overall, this experiment suggests that both PCP and nicotine alter 50 kHz vocalizations, in different ways. PCP acutely decreased 50 kHz vocalizations while repeated exposure to nicotine led to higher levels of 50 kHz vocalizations. Specific frequency-modulated vocalizations were lessened by nicotine and PCP. This is the first study to report differences in subtypes of vocalizations due to PCP exposure. Given the complexities of the PCP model of schizophrenia symptoms, examination of different call profiles of animals in this model is warranted and further examination of the interaction of antipsychotics with this effect would be illuminating. Vocalization subtypes resembling those seen in the 50 kHz categories were also noted in the 22 kHz range. Given that these vocalizations were increased after nicotine treatment, future research on the potential mechanisms and behavioral significance of these vocalizations is necessary. Finally, this study lends credence to the idea that examining behaviors after discontinuation of PCP may better model discrepancies in reward and reinforcement seen in patients with schizophrenia that might not be uncovered during acute and chronic treatment.

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References

Abdul-Monim Z, Reynolds GP, Neill JC. The atypical antipsychotic ziprasidone, but not haloperidol, improves phencyclidine-induced cognitive deficits in a reversal learning task in the rat. Journal of Psychopharmacology. 2003; 17(1):57–66. [PubMed: 12680740]

Abdul-Monim Z, Neill JC, Reynolds GP. Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat. Journal of Psychopharmacology. 2007; 21:198–205. [PubMed: 17329300]

Ahnallen CG, Liverant GI, Gregor KL, Kamholz BW, Levitt JJ, Gulliver SB, et al. The relationship between reward-based learning and nicotine dependence in smokers with schizophrenia. Psychiatry Res. 2012; 196:9–14. [PubMed: 22342123]

Ahrens AM, Ma ST, Maier EY, Duvauchelle CL, Schallert T. Repeated intravenous amphetamine exposure: rapid and persistent sensitization of 50-kHz ultrasonic trill calls in rats. Behav Brain Res. 2009; 197:205–209. [PubMed: 18809437]

Ahrens AM, Nobile CW, Page LE, Maier EY, Duvauchelle CL, Schallert T. Individual differences in the conditioned and unconditioned rat 50-kHz ultrasonic vocalizations elicited by repeated amphetamine exposure. Psychopharmacology (Berl). 2013; 229:687–700. [PubMed: 23700082]

Balfour DJ, Ridley DL. The effects of nicotine on neural pathways implicated in depression: a factor in nicotine addiction? Pharmacol Biochem Behav. 2000; 66:79–85. [PubMed: 10837846]

Barker DJ, Root DH, Ma S, Jha S, Megehee L, Pawlak AP, West MO. Dose-dependent differences in short ultrasonic vocalizations emitted by rats during cocaine self-administration. Psychopharmacology. 2010; 211(4):435–442. [PubMed: 20571780]

Barrett ST, Bevins RA. A quantitative analysis of the reward-enhancing effects of nicotine using reinforcer demand. Behav Pharmacol. 2012; 23:781–789. [PubMed: 23080311]

Barrett ST, Odum AL. The effects of repeated exposure on the reward-enhancing effects of nicotine. Behav Pharmacol. 2011; 22:283–290. [PubMed: 21562409]

Berlyne DE, Koenig ID. Some possible parameters of photic reinforcement. J Comp Physiol Psychol. 1965; 60:276–280. [PubMed: 5832361]

Bevins RA, Besheer J, Pickett KS. Nicotine-conditioned locomotor activity in rats: Dopaminergic and GABAergic influences on conditioned expression. Pharmacology, Biochemistry and Behavior. 2001; 68:135–145.

Blanchard RJ, Blanchard DC, Agullana R, Weiss SM. Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. Physiology & behavior. 1991; 50(5): 967–972. [PubMed: 1805287]

Boulay D, Ho-Van S, Bergis O, Avenet P, Griebel G. Phencyclidine decreases tickling-induced 50-kHz ultrasound vocalizations in juvenile rats: a putative model of the negative symptoms of schizophrenia? Behav Pharmacol. 2013

Browning JR, Browning DA, Maxwell AO, Dong Y, Jansen HT, Panksepp J, et al. Positive affective vocalizations during cocaine and sucrose self-administration: a model for spontaneous drug desire in rats. Neuropharmacology. 2011; 61:268–275. [PubMed: 21530553]

Brunelli SA, Nie R, Whipple C, Winiger V, Hofer MA, Zimmerberg B. The effects of selective breeding for infant ultrasonic vocalizations on play behavior in juvenile rats. Physiol Behav. 2006; 87:527–536. [PubMed: 16488454]

Buda M, Tsuang MT, Fleming JA. Causes of death in DSM-III schizophrenics and other psychotics (atypical group). A comparison with the general population. Arch Gen Psychiatry. 1988; 45:283–285. [PubMed: 3341883]

Burgdorf J, Panksepp J. The neurobiology of positive emotions. Neurosci Biobehav Rev. 2006; 30:173–187. [PubMed: 16099508]

Burgdorf J, Wood PL, Kroes RA, Moskal JR, Panksepp J. Neurobiology of 50-kHz ultrasonic vocalizations in rats: electrode mapping, lesion, and pharmacology studies. Behav Brain Res. 2007; 182:274–283. [PubMed: 17449117]

Caggiula AR, Donny EC, Chaudhri N, Perkins KA, Evans-Martin FF, Sved AF. Importance of nonpharmacological factors in nicotine self-administration. Physiol Behav. 2002; 77:683–687. [PubMed: 12527019]

Caggiula AR, Donny EC, Palmatier MI, Liu X, Chaudhri N, Sved AF. The role of nicotine in smoking: a dual-reinforcement model. Nebr Symp Motiv. 2009; 55:91–109. [PubMed: 19013940]

Center for Disease Control. [Retrieved May 1, 2014] Adult Cigarette Smoking in the United States: Current Estimates. (2014, February 14). Centers for Disease Control and Prevention. 2014. 2014 from http://www.cdc.gov/tobacco/data_statistics/fact_sheets/adult_data/cig_smoking/index.htm

Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, et al. Operant responding for conditioned and unconditioned reinforcers in rats is differentially enhanced by the primary reinforcing and reinforcement-enhancing effects of nicotine. Psychopharmacology (Berl). 2006; 189:27–36. [PubMed: 17019569]

Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, et al. Self-administered and noncontingent nicotine enhance reinforced operant responding in rats: impact of nicotine dose and reinforcement schedule. Psychopharmacology (Berl). 2007; 190:353–362. [PubMed: 16847680]

Donny EC, Chaudhri N, Caggiula AR, Evans-Martin FF, Booth S, Gharib MA, et al. Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. Psychopharmacology (Berl). 2003; 169:68–76. [PubMed: 12774186]

Ellenbroek BA, Cools AR. Animal models for the negative symptoms of schizophrenia. Behav Pharmacol. 2000; 11:223–233. [PubMed: 11103877]

Francis RL. 22-kHz calls by isolated rats. Nature. 1977; 265:236–238. [PubMed: 834267]

Glow PH, Russell A. The effects of a dexamphetamine-amylobarbitone sodium mixture on the reward value of different sensory changes. Psychopharmacologia. 1975; 41:181–185. [PubMed: 1153606]

Gopalaswamy AK, Morgan R. Smoking in chronic schizophrenia. Br J Psychiatry. 1986; 149:523. [PubMed: 3814944]

Hennekens CH, Hennekens AR, Hollar D, Casey DE. Schizophrenia and increased risks of cardiovascular disease. Am Heart J. 2005; 150:1115–1121. [PubMed: 16338246]

Jelen P, Soltysik S, Zagrodzka J. 22-kHz ultrasonic vocalization in rats as an index of anxiety but not fear: behavioral and pharmacological modulation of affective state. Behav Brain Res. 2003; 141:63–72. [PubMed: 12672560]

Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology. 1999; 20:201–225. [PubMed: 10063482]

Jentsch JD, Tran A, Le D, Youngren KD, Roth RH. Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. neuropsychopharmacology. 1997; 17(2):92–99. [PubMed: 9252984]

Knutson B, Burgdorf J, Panksepp J. Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. J Comp Psychol. 1998; 112:65–73. [PubMed: 9528115]

Kumari V, Postma P. Nicotine use in schizophrenia: the self medication hypotheses. Neurosci Biobehav Rev. 2005; 29:1021–1034. [PubMed: 15964073]

Liu X, Palmatier MI, Caggiula AR, Donny EC, Sved AF. Reinforcement enhancing effect of nicotine and its attenuation by nicotinic antagonists in rats. Psychopharmacology (Berl). 2007; 194:463–473. [PubMed: 17616849]

Machold RP. Loss of rostral brainstem cholinergic activity results in decreased ultrasonic vocalization behavior and altered sensorimotor gating. Behav Brain Res. 2013; 256:51–55. [PubMed: 23810416]

Maier EY, Abdalla M, Ahrens AM, Schallert T, Duvauchelle CL. The missing variable: ultrasonic vocalizations reveal hidden sensitization and tolerance-like effects during long-term cocaine administration. Psychopharmacology (Berl). 2012; 219:1141–1152. [PubMed: 21870038]

Mead A, Li M, Kapur S. Clozapine and olanzapine exhibit an intrinsic anxiolytic property in two conditioned fear paradigms: contrast with haloperidol and chlordiazepoxide. Pharmacol Biochem Behav. 2008; 90(4):551–562. [PubMed: 18547622]

Mortensen PB, Juel K. Mortality and causes of death in schizophrenic patients in Denmark. Acta Psychiatr Scand. 1990; 81:372–377. [PubMed: 2188483]

Mouri A, Noda Y, Enomoto T, Nabeshima T. Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. Neurochem Int. 2007; 51:173–184. [PubMed: 17669558]

Mu P, Fuchs T, Saal DB, Sorg BA, Dong Y, Panksepp J. Repeated cocaine exposure induces sensitization of ultrasonic vocalization in rats. Neurosci Lett. 2009; 453:31–35. [PubMed: 19429010]

Nabeshima T, Hiramatsu M, Amano M, Furukawa H, Kameyama T. Chronic phencyclidine increases methionine-enkephalin level in mouse striatum. Neurosci Lett. 1983; 37:69–74. [PubMed: 6877660]

Neill JC, Barnes S, Cook S, Grayson B, Idris NF, McLean SL, et al. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. Pharmacol Ther. 2010; 128:419–432. [PubMed: 20705091]

Neill JC, Harte MK, Haddad PM, Lydall ES, Dwyer DM. Acute and chronic effects of NMDA receptor antagonists in rodents, relevance to negative symptoms of schizophrenia: A translational link to humans. Eur Neuropsychopharmacol. 2013; 24:822–835. [PubMed: 24287012]

Palmatier MI, Evans-Martin FF, Hoffman A, Caggiula AR, Chaudhri N, Donny EC, et al. Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. Psychopharmacology (Berl). 2006; 184:391–400. [PubMed: 16249908]

Palmatier MI, Matteson GL, Black JJ, Liu X, Caggiula AR, Craven L, et al. The reinforcement enhancing effects of nicotine depend on the incentive value of non-drug reinforcers and increase with repeated drug injections. Drug Alcohol Depend. 2007; 89:52–59. [PubMed: 17240084]

Panksepp J, Burgdorf J. 50-kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: effects of social housing and genetic variables. Behav Brain Res. 2000; 115:25–38. [PubMed: 10996405]

Pich EM, Pagliusi SR, Tessari M, Talabot-Ayer D, Hooft van Huijsduijnen R, Chiamulera C. Common neural substrates for the addictive properties of nicotine and cocaine. Science. 1997; 275:83–86. [PubMed: 8974398]

Raiff BR, Dallery J. Effects of acute and chronic nicotine on responses maintained by primary and conditioned reinforcers in rats. Exp Clin Psychopharmacol. 2006; 14:296–305. [PubMed: 16893272]

Raiff BR, Dallery J. The generality of nicotine as a reinforcer enhancer in rats: effects on responding maintained by primary and conditioned reinforcers and resistance to extinction. Psychopharmacology (Berl). 2008; 201:305–314. [PubMed: 18695928]

Raiff BR, Dallery J. Responding maintained by primary reinforcing visual stimuli is increased by nicotine administration in rats. Behav Processes. 2009; 82:95–99. [PubMed: 19615619]

Sales GD. Ultrasound and aggressive behaviour in rats and other small mammals. Anim Behav. 1972; 20:88–100. [PubMed: 4677167]

Saha S, Chant D, McGrath J. A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? Arch Gen Psychiatry. 2007; 64:1123–1131. [PubMed: 17909124]

Schwarting RK, Jegan N, Wohr M. Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats. Behav Brain Res. 2007; 182:208–222. [PubMed: 17367876]

Simola N, Fenu S, Costa G, Pinna A, Plumitallo A, Morelli M. Pharmacological characterization of 50-kHz ultrasonic vocalizations in rats: comparison of the effects of different psychoactive drugs and relevance in drug-induced reward. Neuropharmacology. 2012; 63:224–234. [PubMed: 22465816]

Simola N, Frau L, Plumitallo A, Morelli M. Direct and long-lasting effects elicited by repeated drug administration on 50-kHz ultrasonic vocalizations are regulated differently: implications for the study of the affective properties of drugs of abuse. The International Journal of Neuropsychopharmacology. 2014; 17(03):429–441. [PubMed: 24138707]

Smith RC, Singh A, Infante M, Khandat A, Kloos A. Effects of cigarette smoking and nicotine nasal spray on psychiatric symptoms and cognition in schizophrenia. Neuropsychopharmacology. 2002; 27:479–497. [PubMed: 12225705]

Stevenson CW, Goodwin PE, Tunstall B, Spicer CH, Marsden CA, Mason R. Neonatal maternal separation alters reward-related ultrasonic vocalizations in rat dams. Behav Brain Res. 2009; 200:232–236. [PubMed: 19378466]

Sun T, He W, Hu G, Li M. Anxiolytic-like property of risperidone and olanzapine as examined in multiple measures of fear in rats. Pharmacol Biochem Behav. 2010; 95(3):298–307. [PubMed: 20167232]

Taracha E, Kaniuga E, Chrapusta SJ, Maciejak P, Sliwa L, Hamed A, et al. Diverging frequency-modulated 50-kHz vocalization, locomotor activity and conditioned place preference effects in rats given repeated amphetamine treatment. Neuropharmacology. 2014; 83C:128–136. [PubMed: 24769001]

Thiel KJ, Sanabria F, Neisewander JL. Synergistic interaction between nicotine and social rewards in adolescent male rats. Psychopharmacology (Berl). 2009; 204:391–402. [PubMed: 19224200]

Tonoue T, Ashida Y, Makino H, Hata H. Inhibition of shock-elicited ultrasonic vocalization by opioid peptides in the rat: a psychotropic effect. Psychoneuroendocrinology. 1986; 11:177–184. [PubMed: 2428074]

Tunstall B, Beckett S, Mason R. Ultrasonic vocalisations explain unexpected effects on pre-pulse inhibition responses in rats chronically pre-treated with phencyclidine. Behav Brain Res. 2009; 202:184–191. [PubMed: 19463700]

Vivian JA, Miczek KA. Diazepam and gepirone selectively attenuate either 20–32 or 32–64 kHz ultrasonic vocalizations during aggressive encounters. Psychopharmacology. 1993a; 112(1):66–73. [PubMed: 7871011]

Vivian JA, Miczek KA. Morphine attenuates ultrasonic vocalization during agonistic encounters in adult male rats. Psychopharmacology. 1993b; 111(3):367–375. [PubMed: 7870976]

White NR, Cagiano R, Moises AU, Barfield RJ. Changes in mating vocalizations over the ejaculatory series in rats (Rattus norvegicus). J Comp Psychol. 1990; 104:255–262. [PubMed: 2225763]

Wohr M, Borta A, Schwarting RK. Overt behavior and ultrasonic vocalization in a fear conditioning paradigm: a dose-response study in the rat. Neurobiol Learn Mem. 1977; 84:228–240. [PubMed: 16115784]

Wohr M, Houx B, Schwarting RK, Spruijt B. Effects of experience and context on 50-kHz vocalizations in rats. Physiol Behav. 2008; 93:766–776. [PubMed: 18191963]

Wright JM, Gourdon JC, Clarke PB. Identification of multiple call categories within the rich repertoire of adult rat 50-kHz ultrasonic vocalizations: effects of amphetamine and social context. Psychopharmacology (Berl). 2010; 211:1–13. [PubMed: 20443111]

Yee N, Schwarting RK, Fuchs E, Wohr M. Juvenile stress potentiates aversive 22-kHz ultrasonic vocalizations and freezing during auditory fear conditioning in adult male rats. Stress. 2012; 15:533–544. [PubMed: 22150360]

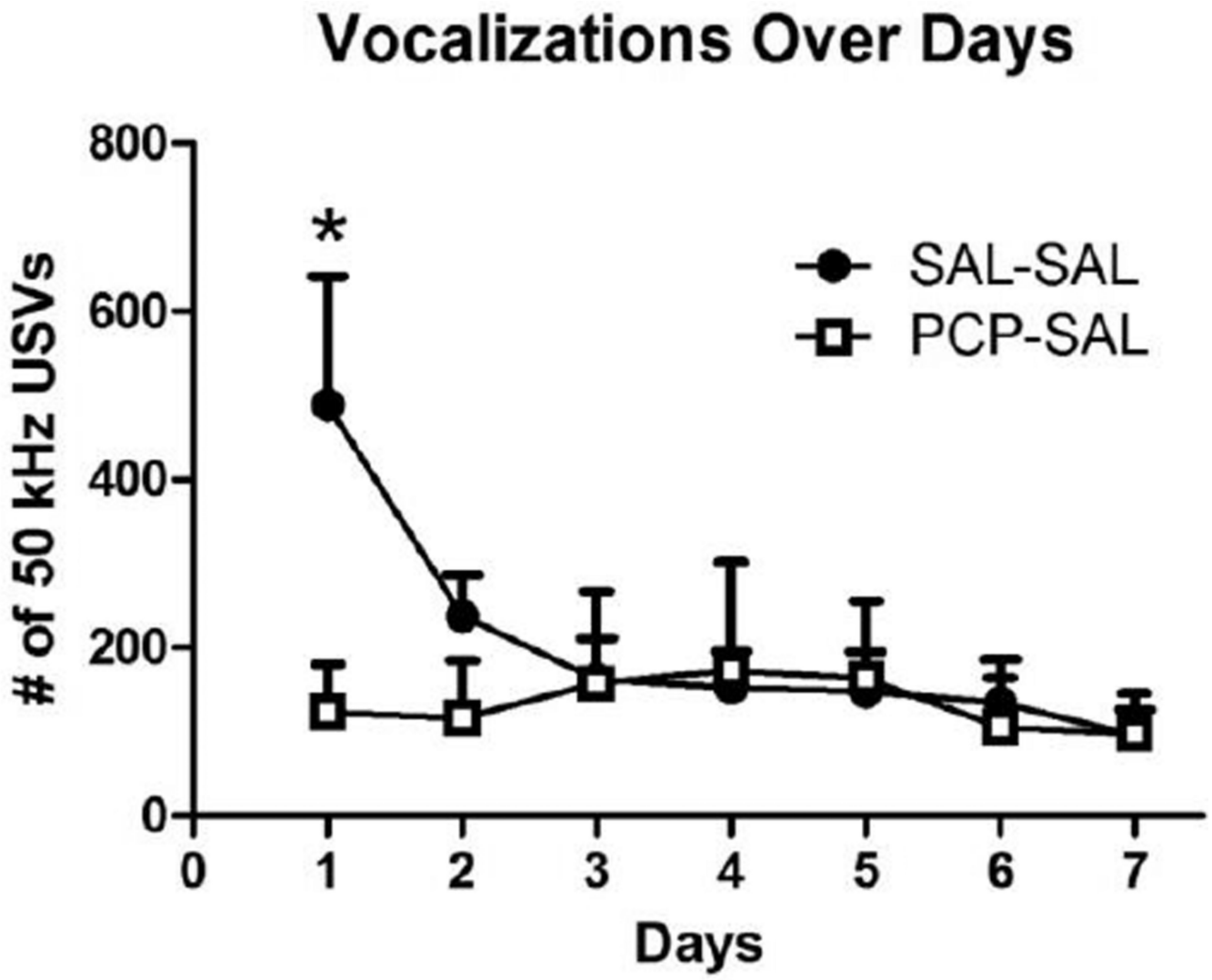

Figure 1.

PCP and SAL group 50 kHz vocalizations (mean+ SEM) by day. The PCP-SAL group emitted fewer 50 kHz vocalizations in comparison to the SAL-SAL group on the first day according to LSD tests * $p<0.05$, n=7 and n=8 respectively

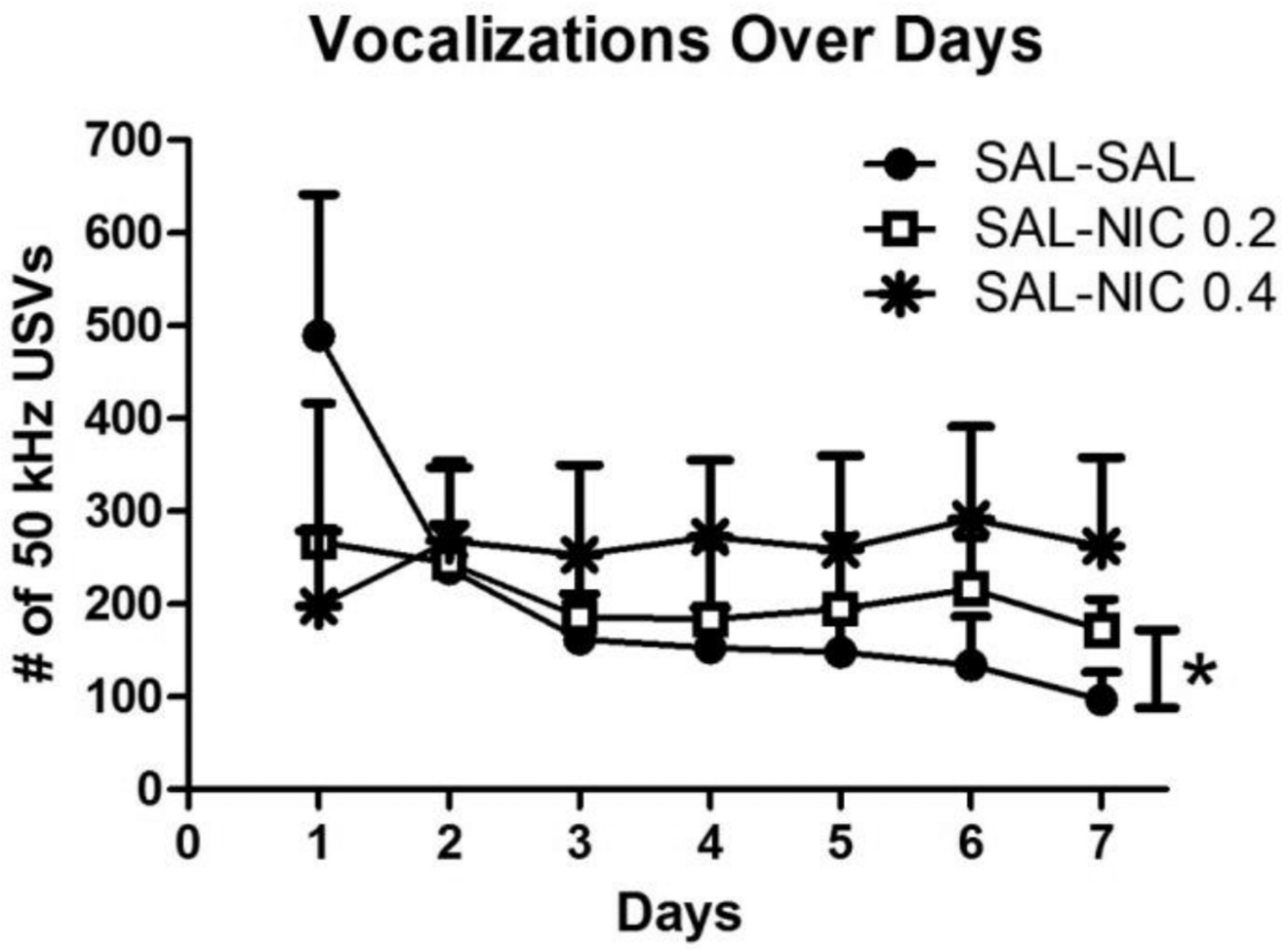

Figure 2.

Effect of nicotine (mean + SEM) on 50 kHz vocalizations over all days of drug testing. The low dose nicotine group emitted more 50 kHz vocalizations than the SAL-SAL group. * $p<0.05$. SAL-SAL, n=7/group; nicotine, n=8/group.

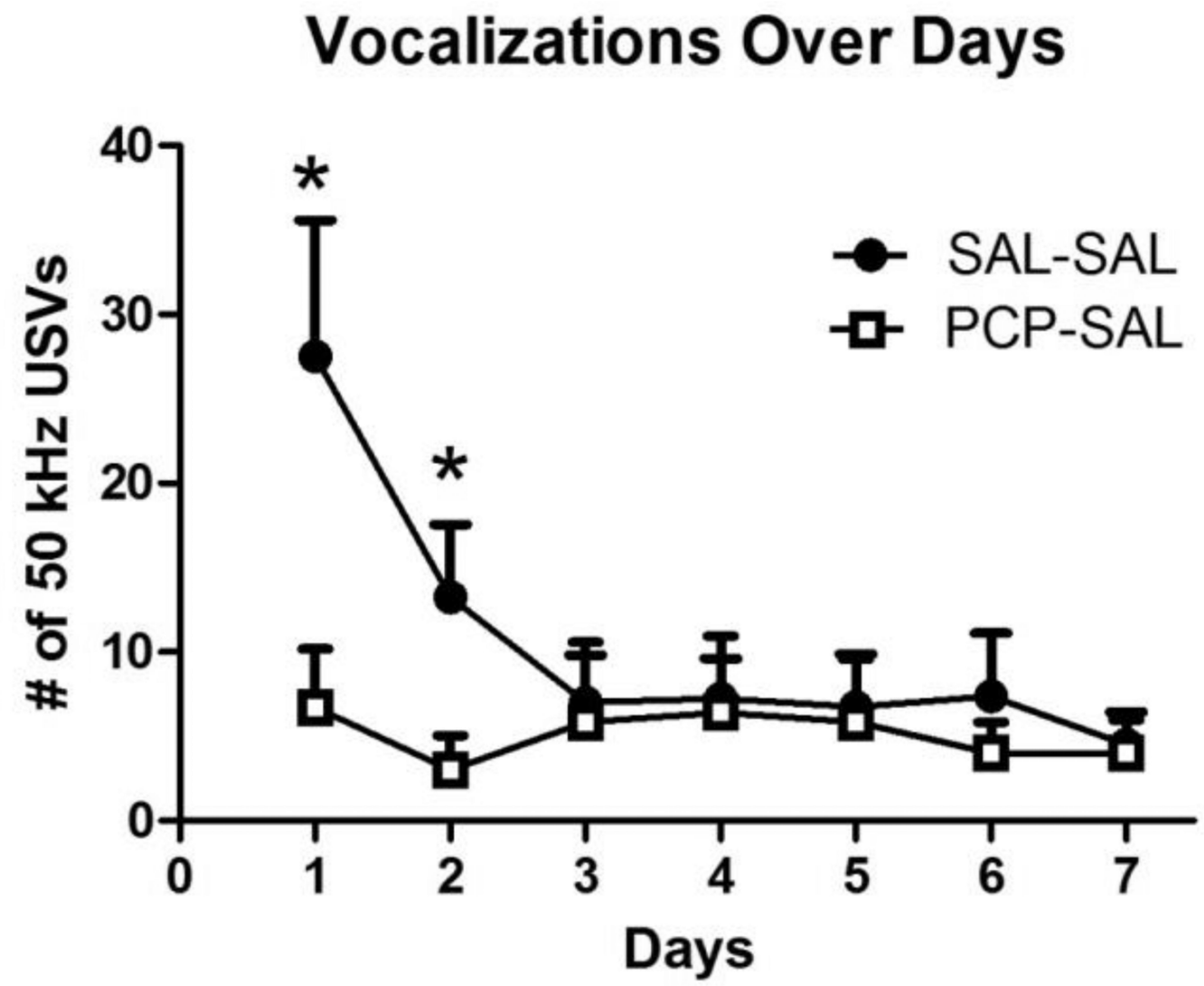

Figure 3.

Effect of PCP (mean ± SEM) on 50 kHz vocalizations emitted during light stimuli, over days of drug testing. The PCP-SAL group made fewer vocalizations than the SAL-SAL group on the first and second day according to LSD tests. * $p<0.05$.

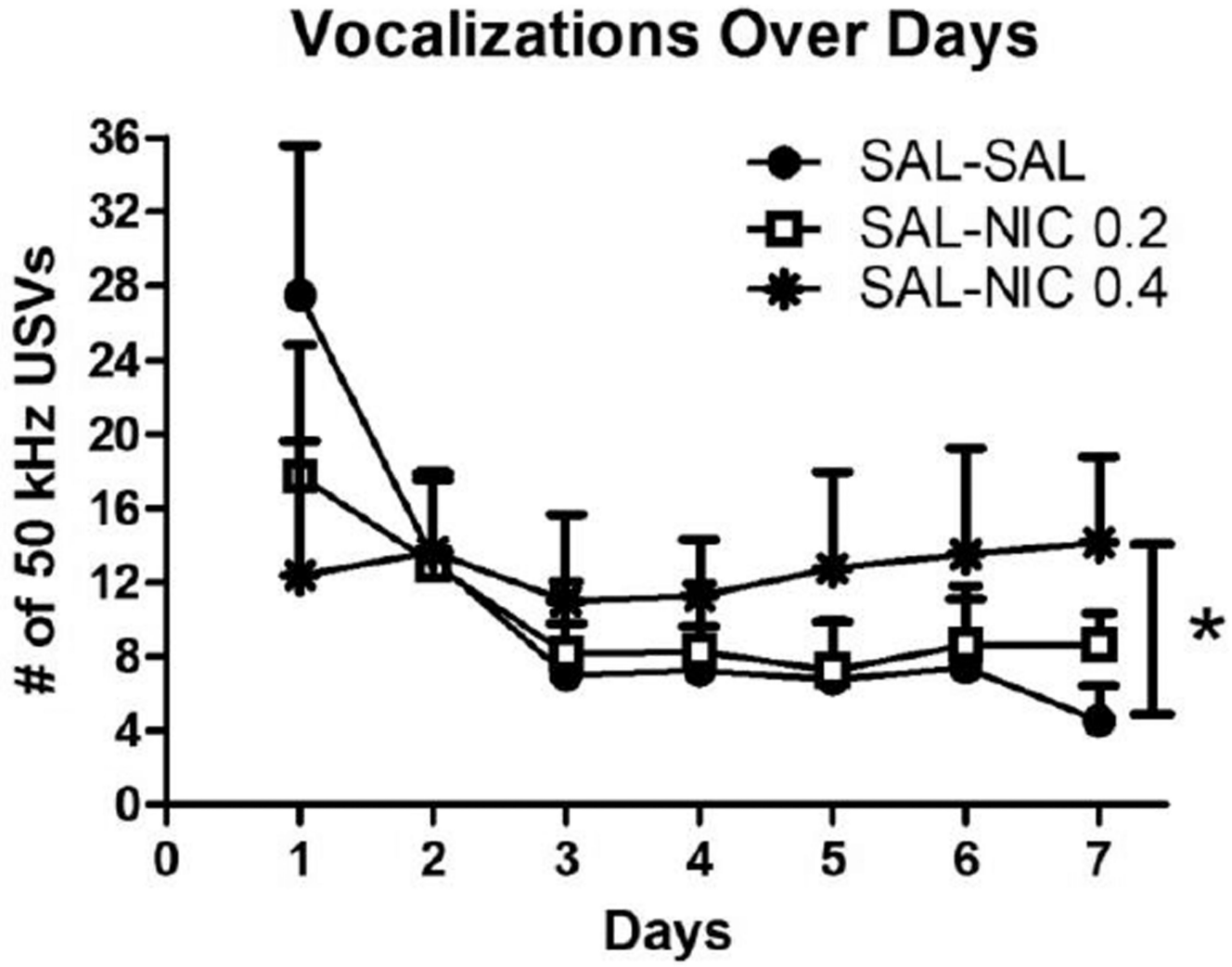

Figure 4.

Effect of nicotine (mean + SEM) on 50 kHz vocalizations emitted during light stimuli over drug testing days. The low dose nicotine group and high dose nicotine group emitted more 50 kHz vocalizations than the SAL-SAL group. * $p<0.05$.

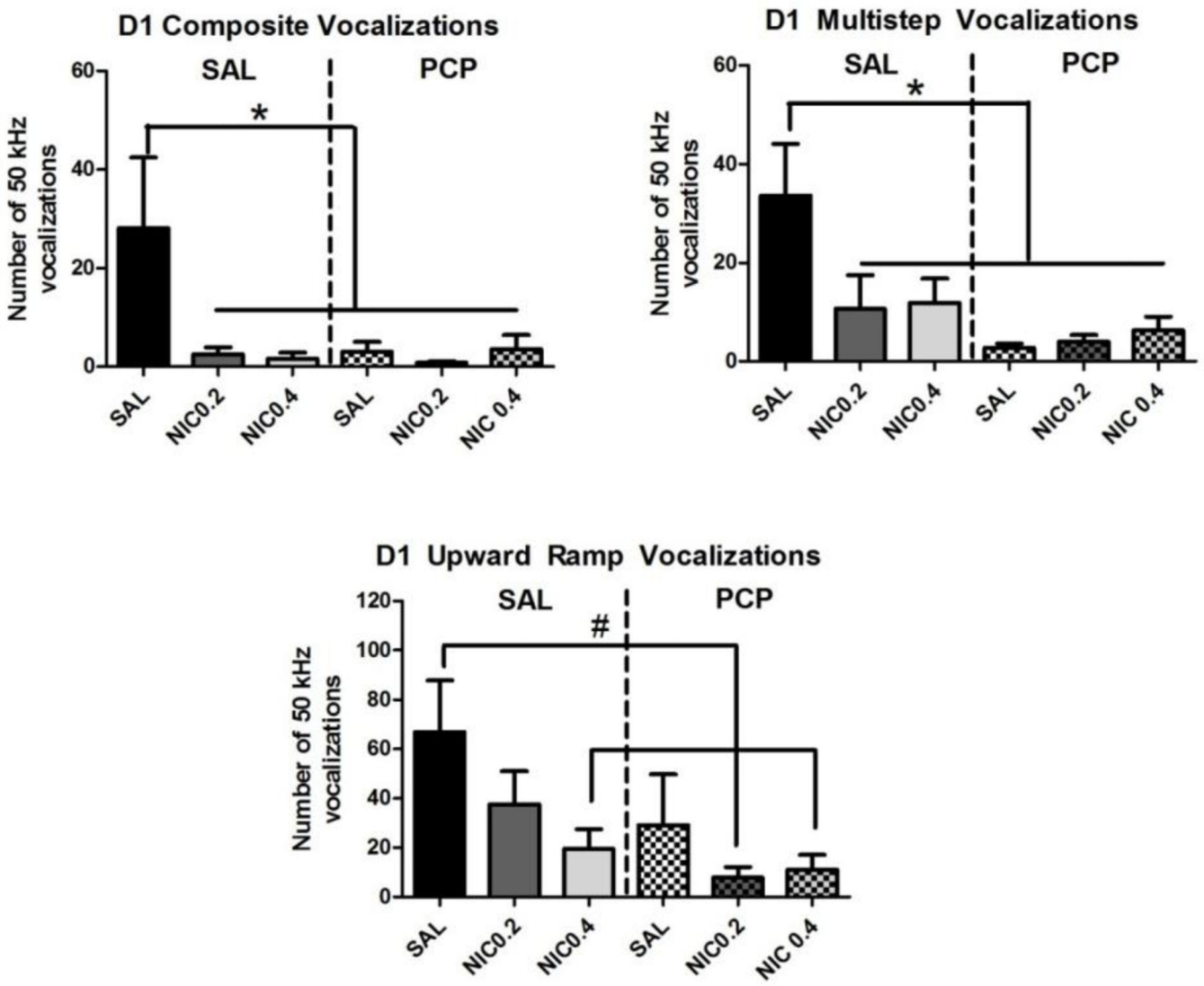

Figure 5.

Differences between groups on the first drug session in subtypes of 50 kHz vocalizations (mean + SEM). SAL-SAL had higher composite and multistep vocalizations than all other groups and groups that had previously received high dose nicotine (0.4 mg/kg) had lower upward ramp vocalizations. $^{\#}p=0.50$; * $p<0.05$. n= 7 or 8/ group.

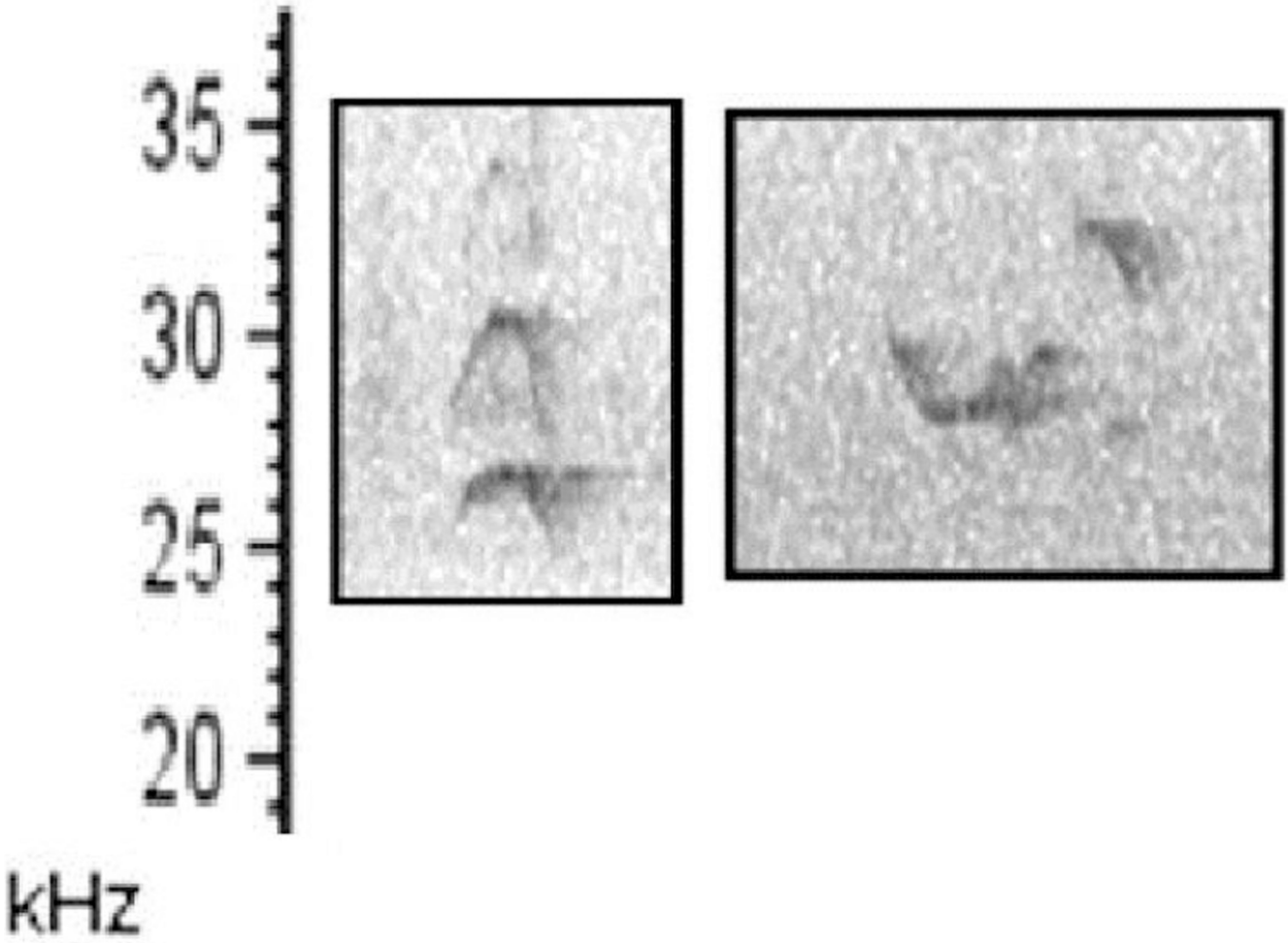

Figure 6.
Examples of vocalizations that look like subtypes of 50 kHz vocalizations but were within the range of 22 kHz vocalizations.

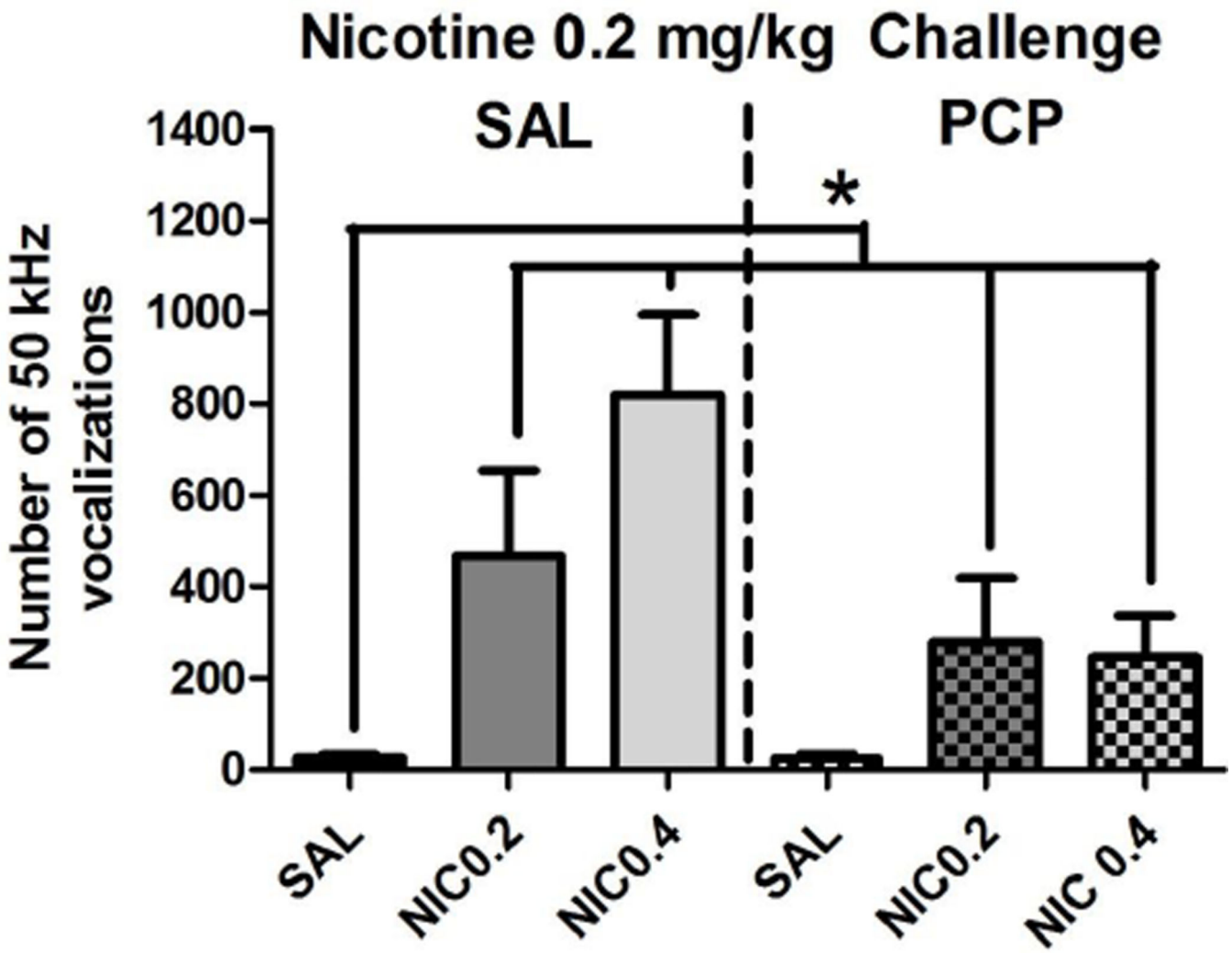

Figure 7.

Overall 50 kHz vocalizations (mean + SEM) on the low -dose nicotine challenge day, separated by group. Rats that had previously received any dose of nicotine had higher vocalizations than the SAL-SAL group. * $p<0.05$

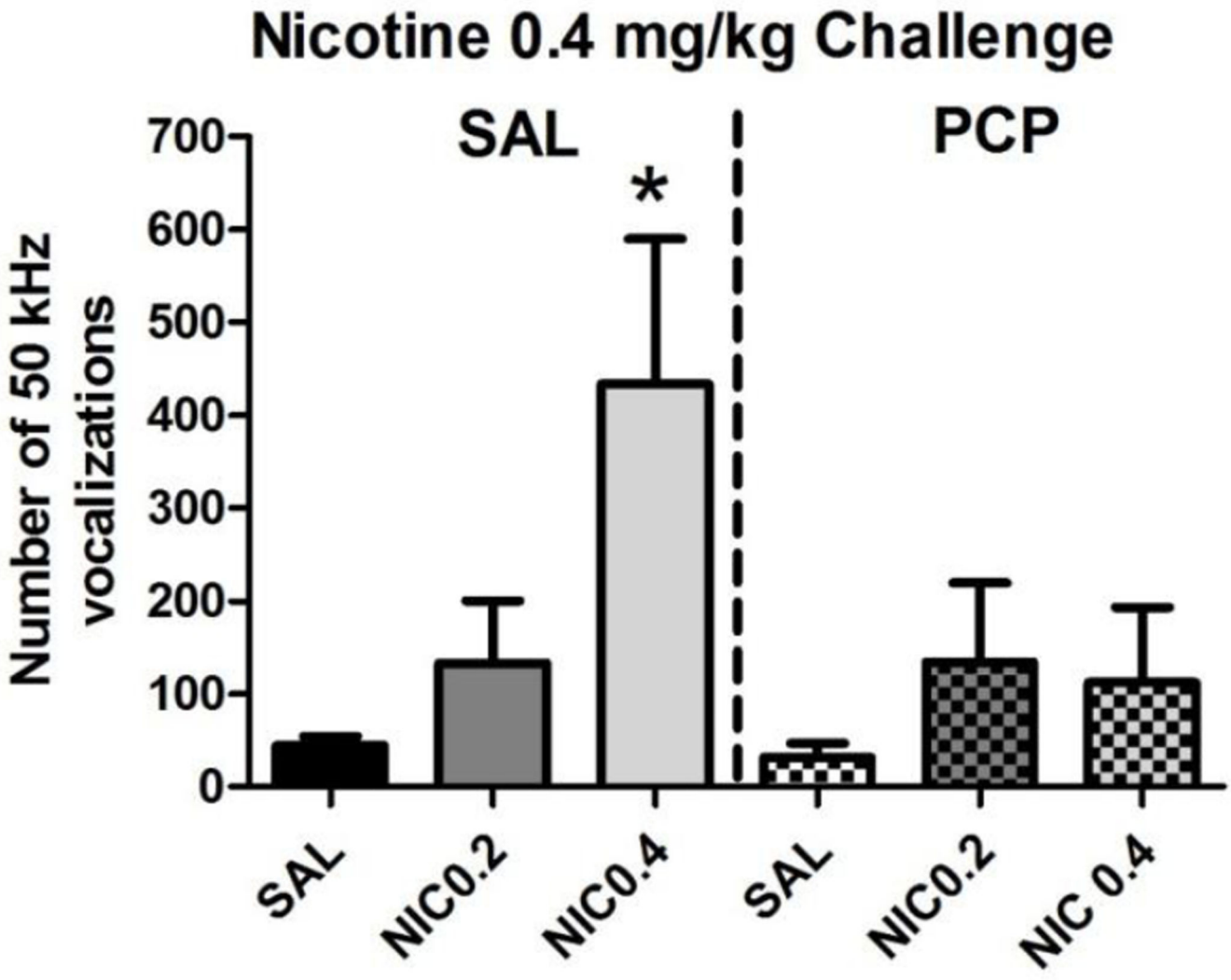

Figure 8.

Overall 50 kHz vocalizations (mean + SEM) on the high-dose nicotine challenge day, separated by group. Only rats that had previously received SAL-NIC 0.4 had higher vocalizations than the SAL-SAL group. * $p<0.05$

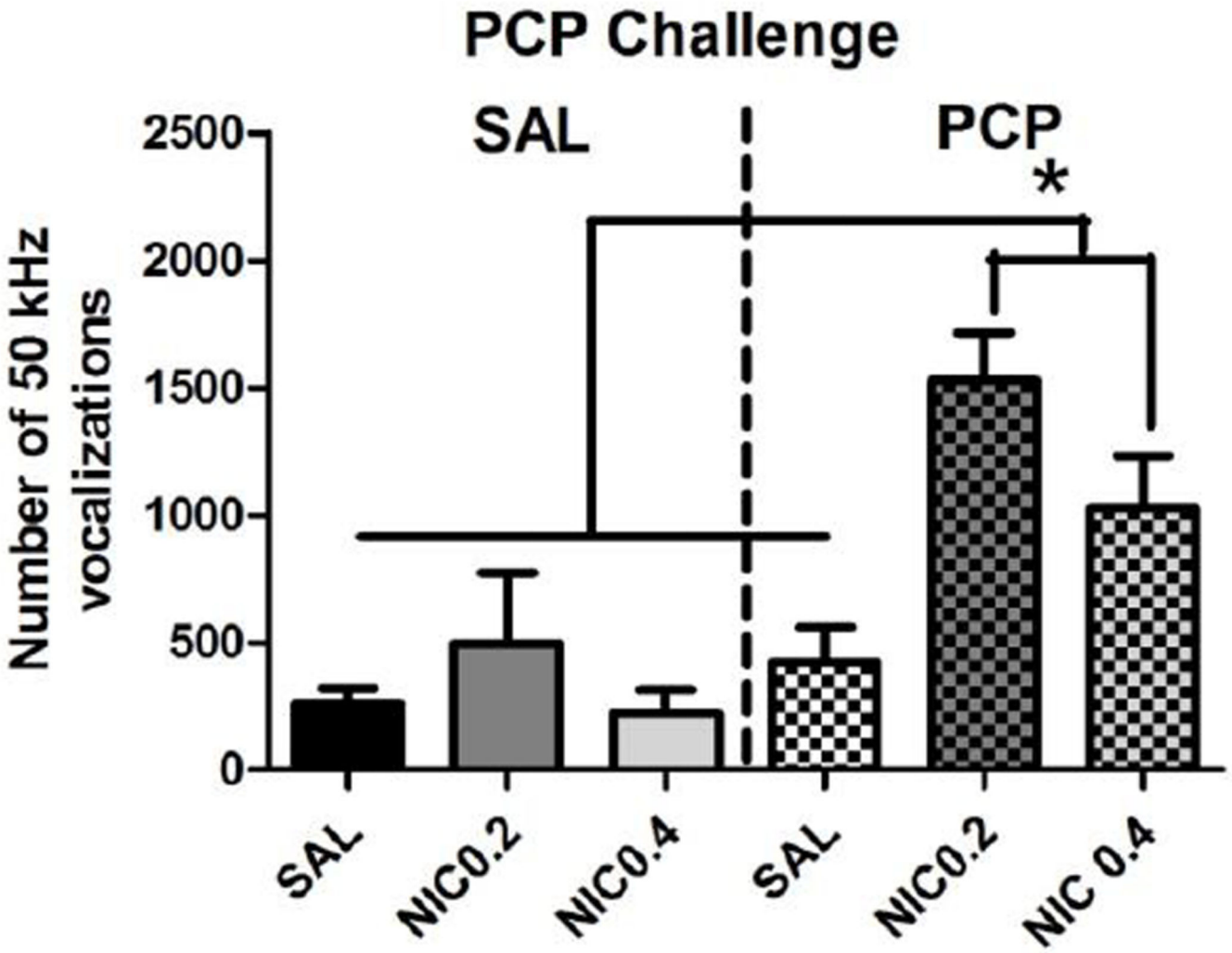

Figure 9.

Overall 50 kHz vocalizations (mean + SEM) on the PCP challenge day, separated by group. Groups that had previously received both nicotine and PCP (either dose) had higher vocalizations than the SAL-SAL and PCP-SAL groups. * $p<0.05$