Published in final edited form as:

Behav Neurosci. 2018 August ; 132(4): 269–283. doi:10.1037/bne0000258.

Noradrenergic receptor modulation influences the acoustic parameters of pro-social rat ultrasonic vocalizations

Laura M. Grant^{1,2}, Kelsey J. Barth¹, Cagla Muslu¹, Cynthia A. Kelm-Nelson¹, Vaishali P. Bakshi^{3,4}, and Michelle R. Ciucci^{1,2,4}

¹Department of Surgery, Division of Otolaryngology-Head and Neck Surgery, University of Wisconsin-Madison, Madison, WI, USA

²Department of Communication Sciences and Disorders, University of Wisconsin-Madison, Madison, WI, USA

³Department of Psychiatry, University of Wisconsin-Madison, Madison, WI, USA

⁴Neuroscience Training Program, University of Wisconsin-Madison, Madison, WI, USA

Abstract

Rats produce high rates of ultrasonic vocalizations (USVs) in social situations; these vocalizations are influenced by multiple neurotransmitter systems. Norepinephrine (NE) plays a significant role in vocalization biology; however, the contribution of NE to normal, pro-social vocal control has not been well established in the rat. To address this, we used NE adrenoceptor agonists (Cirazoline, Clonidine) and antagonists (Prozasin, Atipamezole, Propranolol) to quantify the contribution of specific alpha-1, alpha-2, and beta NE receptors to USV parameters in male Long Evans rats during semi-naturalistic calling. We found that multiple USV acoustic variables (intensity, bandwidth, duration, peak frequency, and call profile) are modified by alterations in NE signaling. Very generally, agents that increased NE neurotransmission (Atipamezole) or activated alpha-1 receptors (Cirazoline), led to an increase in intensity and duration, respectively. Agents that decreased NE neurotransmission (Clonidine) or blocked alpha-1 receptors (Prazosin) reduced call rate, intensity, and bandwidth. However, the beta-receptor antagonist, Propranolol, was associated with increased call rate, duration, and intensity. Limb motor behaviors were largely unaffected by any drug, with the exception of Clonidine. Higher doses of Clonidine significantly reduced gross motor, grooming, and feeding behavior. These results confirm the involvement of NE transmission in vocal control in the rat, and suggest that this USV model is useful for studying the neuropharmacology of behavioral measures that may have implications for disease states, such as Parkinson disease.

Keywords

Norepinephrine; noradrenaline; ultrasonic vocalization; rat

Corresponding Author: Michelle R. Ciucci, Ph.D., Department of Surgery, Division of Otolaryngology, 1300 University Avenue, 438 Medical Sciences Center, University of Wisconsin-Madison, Madison, WI 53706 USA, Phone: 1-608-262-6122, Fax: 1-608-262-6356, Ciucci@surgery.wisc.edu.

Financial Disclosure/Conflict of Interest: None

1. Introduction

Noradrenergic circuitry is responsible for maintaining and regulating behaviors critical to an animal's environment. Specifically, vocalizations are complex behaviors that are modulated by multiple neural circuits (Brudzynski, 2010; Brudzynski, Komadoski and St Pierre, 2012; Scardochio and Clarke, 2013; Wright, Dobosiewicz and Clarke, 2013; Costa, Morelli and Simola, 2015; Hamed, Szyndler, Taracha, Turzynska, Sobolewska et al., 2015; Wohr, Rippberger, Schwarting and van Gaalen, 2015; Wohr, van Gaalen and Schwarting, 2015; Hamed, Daszczuk, Kursa, Turzynska, Sobolewska et al., 2016; Simola, Costa and Morelli, 2016). Rodents produce ultrasonic vocalizations (USV) and provide a unique model system to study the contribution of the noradrenergic system to vocalization behavior. While this quantifiable form of social communication has been reasonably well studied, little is known about how specific noradrenergic receptor agonism and antagonism influence specific acoustic parameters relevant to vocal communication deficits observed in humans and transgenic/genetic rodent models of disease.

Norepinephrine (NE) and its receptors are widely distributed throughout the central nervous system, where they are thought to be neuromodulatory, regulating attention, vigilance, signal-noise-ratio, and broadly influencing a wide range of behavioral functions including vocalizations (Barclay, Harding and Waterman, 1996; Castelino and Schmidt, 2010). There are three main receptors for NE: α_1 -adrenergic receptor (AR), α_2 -AR, and β -AR (Rohrer and Kobilka, 1998). α_1 -AR and β -AR can be found post-synaptically throughout the central and peripheral nervous system, however the α_2 -AR is primarily located pre-synaptically and functions as an autoreceptor that, when stimulated, reduces NE release (Fornai, Alessandri, Fascetti, Vaglini and Corsini, 1995; Rommelfanger and Weinshenker, 2007), although these categorizations are not absolute and more complex roles for α_2 receptors have been found. This system is conserved across vertebrates (Smeets and Gonzalez, 2000), acts to coordinate and activate multiple brain activities (Berridge and Waterhouse, 2003; Marien, Colpaert and Rosenquist, 2004; Aston-Jones and Cohen, 2005; Chamberlain and Robbins, 2013), and is well positioned to influence vocalizations.

Male adult rats emit 50 kHz frequency-modulated ultrasonic vocalizations in a variety of social situations, including in response to female conspecifics. Rats produce several types of USVs that can be classified by complexity and frequency characteristics of the waveform (Knutson, Burgdorf and Panksepp, 2002; Ciucci, Ma, Fox, Kane, Ramig et al., 2007; Portfors, 2007; Wöhr, Houx, Schwarting and Spruijt, 2008; Ciucci and Connor, 2009). USV produced in a socio-sexual paradigm are composed of short, constant frequency calls (simple calls) as well as complex frequency modulated subtypes (McGinnis and Vakulenko, 2003; Brudzynski, 2005; Costantini and D'Amato F, 2006; Burgdorf, Wood, Kroes, Moskal and Panksepp, 2007; Ciucci, Ma, Fox, Kane, Ramig et al., 2007; Wöhr, Houx, Schwarting and Spruijt, 2008; Ciucci, Ahrens, Ma, Kane, Windham et al., 2009). There is pharmacological evidence that suggests noradrenergic involvement in vocal behavior (Riters and Pawlisch, 2007; Heimovics, Cornil, Ellis, Ball and Riters, 2011; Wright, Dobosiewicz and Clarke, 2012; Wright, Dobosiewicz and Clarke, 2013). For example, administration of the α_2 NE receptor agonist Clonidine increases calling in rat pups (Kehoe and Harris, 1989). Additionally, differential stimulation of alpha and beta adrenergic receptors elicits 50-kHz

vocalizations in amphetamine-induced calling in rats (Wright, Dobosiewicz and Clarke, 2012). While these previous finding suggests a potential role for NE receptor modulation of rodent vocalizations, how NE regulates specific acoustic components of this behavior remains unclear. Moreover, the relative contributions of the three major NE receptor subtypes ($\alpha 1$, $\alpha 2$, β) to acoustic properties of calls have not been determined in a pro-social (induction of conspecific behavioral responses including approach) communication model. Given the potential clinical relevance of vocalization behaviors, particularly in disorders such as Parkinson disease where there are presumed NE system dysfunction (Barclay, Harding and Waterman, 1996; Chamberlain and Robbins, 2013; Grant, Richter, Miller, White, Fox et al., 2014; Grant, Kelm-Nelson, Hilby, Blue, Rajamanickam et al., 2015), it is useful to understand the precise function of NE receptors in promoting and/or reducing different forms of vocalization.

The purpose of this study was to determine how NE ARs modulate specific acoustic parameters in the rat. Specifically, we hypothesized that selectively targeting NE receptors would result in the differential modulation of pertinent acoustic properties of USV including intensity, bandwidth, peak frequency, and duration, in addition to general parameters such as call profile and call rate. We also hypothesized that locomotor and limb behavior would be influenced in a dose-dependent manner. Due to the paucity of information in the literature about NE regulation of vocal behavior, we optimized our paradigm to detect either increases or decreases in the various parameters under study. Thus, we could to determine whether NE AR activity was necessary and/or sufficient for each of these measures.

2. Methods

2.1 Animals, habituation, and experimental overview

Fifty male Long-Evans (Charles River) rats, aged 4–5 months, were used in this study. Rats were randomized into one of five groups ($n=10$ per group) that received three doses of a specific NE receptor drug and vehicle, described below. An additional 8 females, aged 2–12 months were used to elicit USV, and not included in analyses. Prior to all testing, male rats were handled and acclimated to experimental procedures 5 days a week for a total of 2 weeks. Rats were pair-housed in standard polycarbonate cages (17 centimeter [cm] $\times$ 28 cm $\times$ 12 cm) on a reverse 12:12 hour (hr) light cycle. All testing occurred under partial red-light illumination during the dark cycle, as rats are nocturnal. Food and water were available *ad libidum*. All procedures were approved by the University of Wisconsin-Madison School of Medicine and Public Health Animal Care and Use Committee, and are consistent with the NIH guidelines on the Care and Use of Laboratory Animals.

Separate groups of rats were used to test each drug ($n=10$ /drug for a total of $n=50$). Each of the five drugs (agonists or antagonists for $\alpha 1$, $\alpha 2$, or β NE receptors) was administered (intraperitoneal (i.p.)) in a within-subjects design. Thus, each rat received a total of 4 injections (3 doses plus vehicle) in a counterbalanced (Latin square) order. Injections were separated by 3 days for wash-out (Table 1). For each injection, rats were placed individually in the test cage (homecage with bedding) for 30 minutes (min) to acclimate to the testing room. Following this period of acclimation, each rat received an injection and was placed back in the home cage alone for 20–30 min (Table 1), depending on the pharmacokinetic

properties of each drug. Drug doses and pharmacological windows were based on published work (Elghozi, Bianchetti, Morselli and Meyer, 1979; Conway and Jarrott, 1980; Jaillon, 1980; Bialer, Johannessen, Kupferberg, Levy, Perucca et al., 2004; Jaiswal and Mallick, 2009; Wright, Dobosiewicz and Clarke, 2012). Then, locomotor activity was measured, followed immediately by USV testing (also described below). Rats remained isolated from their cage-mates for the duration of injection and testing periods only and remained dually housed for the non-testing portions of the study.

2.2 Drug treatments

Doses, receptor targets, and timings for drug administration are shown in Table 1 and Figure 1. Each of the following drugs was dissolved in 0.9% sterile isotonic saline and injected at a dose of 1 mg/kg: Cirazoline HCL (PubChem CID: 11957512; Sigma Aldrich); Prazosin HCL (PubChem CID: 68546; MP Biomedicals, LLC); Clonidine HCL (PubChem CID: 20179; Sigma Aldrich); Atipamezole (PubChem CID: 71310; Sigma Aldrich); Propranolol hydrochloric acid (HCL) (PubChem CID: 62882; Sigma Aldrich) was prepared in distilled water with 5% DMSO (Sigma Aldrich), and sonicated until dissolved. Drug doses and time course windows were based on published work (Elghozi, Bianchetti, Morselli and Meyer, 1979; Conway and Jarrott, 1980; Jaillon, 1980; Bialer, Johannessen, Kupferberg, Levy, Perucca et al., 2004; Jaiswal and Mallick, 2009; Wright, Dobosiewicz and Clarke, 2012).

2.3 Ultrasonic vocalization recording and analysis

USV were recorded with an ultrasonic microphone with a flat frequency response up to 150 kHz, a working frequency-response range of 10–180 kHz (CM16, Avisoft, Germany), 16-bit resolution and sampled at 250 kHz. The microphone was mounted 15 cm above a standard polycarbonate rat cage. Each male rat was paired with a sexually receptive (estrus) female in the home cage (cage mate temporarily rehoused). After the male demonstrated interest in the female (sniffing, mounting, chasing), the female was removed and male-only vocalizations were recorded for 90 seconds (sec).

Offline acoustic analysis was completed by two raters masked to experimental condition with a customized automated program using SASLab Pro (Avisoft, Germany). Spectrograms were built from each waveform with the frequency resolution set to an FFT of 512 points, a frame size of 100% and flat top window, and the temporal resolution set to display 75 % overlap. Extraneous low frequency noise was removed from the spectrogram using a high pass filter set to exclude noise below 25 kHz (Johnson, Doll, Grant, Ringel, Shier et al., 2011). Variables measured included: latency to the first call (sec), call rate (number of calls/sec; calculated as the total number of calls for the first 60 seconds after calling commenced, divided by 60), overall call profile (% of individual call type; Table 2), and standard deviation of intensity (described below).

To determine call profile, individual calls were classified using a modified version of the 'call profile classification scheme' developed and used previously (Wright, Gourdon and Clarke, 2010; Wright, Dobosiewicz and Clarke, 2012): flat, step, short, ramp, trill, harmonic, frequency modulated (FM), and compound (Figure 2). For analyses of all other acoustic variables, calls were grouped to the broad categories of simple, complex, and compound

(Ciucci, Ma, Fox, Kane, Ramig et al., 2007; Ciucci, Ahrens, Ma, Kane, Windham et al., 2009; Ringel, Basken, Grant and Ciucci, 2013; Grant, Barnett, Doll, Levenson and Ciucci, 2015) (Figure 2). For this study, the following variables were obtained for each rat within the broad category: bandwidth (Hz), peak frequency (kHz), duration (sec), and intensity or “loudness” (decibels [dB]) (Ciucci, Ahrens, Ma, Kane, Windham et al., 2009; Johnson, Doll, Grant, Ringel, Shier et al., 2011; Riede, 2013; Riede, 2014) and are reported as mean and standard error of the mean (SEM).

During recording, rats moved freely around the cage, resulting in variable mouth-to-mic distance that may affect intensity measures. In our experience, the impact of this movement and inherent variability in mouth-to-mic distance is negligible as all rats are tested in the exact same manner and produce calls in all parts of the cage, resulting in an equally heterogeneous sample of call intensity across animals. However, to account for this quantitatively, we calculated the standard deviation of intensity (dB) during each recording session as a gross indication of the range of intensities produced by each rat under each drug condition.

2.4 Locomotor activity and latency to mount

To measure alterations in gross motor function resulting from a drug, locomotor activity was assayed. After drug administration was allowed to reach peak effect (20–30 min), each rat was recorded in their home-cage using a digital camera (Sony HDR-CX210, New York, NY) for 20 min. Videos were analyzed offline by three experienced raters masked to condition for the following variables: total number of cage crossings, total number of rears, time spent grooming (sec), and time spent eating (sec).

Latency to first mount (sec) the female was measured during USV testing to quantify the males' interest in the female. Typically, well-acclimated rats will demonstrate interest and attempt to mount within 1–2 min of exposure to a female. However, occasionally, a rat did not mount within 5 min. This did not happen often, and as all male rats were sexually experienced as part of the study acclimation procedures, had mounted on previous occasions, and showed other signs of interest (chasing, sniffing) the female was removed and vocalizations were recorded.

2.5 Statistical analysis

Separate single-factor (drug dose) repeated measures analyses of variance (ANOVAs) were carried out on each variable for each drug. All data were tested for normality (Shapiro-Wilk test) and equal variances; if the data failed to meet the assumptions for ANOVA, rank or log transformations were used. When significant main effects were seen, post-hoc comparisons were made using Fisher's LSD. The critical level of significance was determined *a priori* at $p < 0.05$. Differences in degrees of freedom reflect cases where the animal did not have the data point.

To determine the degree to which USV and locomotor data were reliably analyzed, an Intraclass Correlation Coefficient (ICC) was calculated on 5% of all the behavioral files analyzed to determine the inter- and intra-rater reliability. A two-way ANOVA was run on

each data set to determine mean squares and degrees of freedom, from which the ICC was computed.

3. Results

Each drug is described separately, ANOVA results for all USV and locomotor variables are detailed below, and means and standard error of the mean (SEM) are reported in the supplemental data (Supplemental Tables 1–5). All significant data are presented in Figures 3–8. The intra- and inter-rater reliability ICC index was above 0.90 for behavioral variables.

3.1 Cirazoline

3.1.1 Locomotor and Latency to mount—There was a main effect of Cirazoline on the number of rears [$F(3,27)=4.29$, $p=0.013$]. The total number of rears was significantly decreased following 0.125 mg/kg ($p=0.019$), 0.25 mg/kg ($p=0.02$), and 0.50 mg/kg ($p=0.002$) compared to vehicle. There were no effects on number of cage crossings, time spent eating, or time spent grooming ($p>0.05$). There was not a main effect of Cirazoline on latency to mount ($p>0.05$). See Supplemental Table 1.

3.1.2 General Vocalization Properties—There was no effect of Cirazoline on call rate, latency to call, or standard deviation of intensity ($p>0.05$; Supplemental Table 2). There was a main effect of drug treatment on the percent of flat calls [$F(3,27)=5.78$, $p=0.003$]. Specifically, compared to vehicle, the percent of flat calls was significantly reduced with 0.125 mg/kg ($p=0.014$), 0.25 mg/kg ($p<0.001$), and 0.50 mg/kg ($p<0.024$) of Cirazoline (data not shown). There were no other significant effects of Cirazoline on call profile ($p>0.05$; data not shown).

3.1.3 Duration—There was a main effect of drug treatment on duration of compound calls [$F(3,27)=4.24$, $p=0.014$]. Duration of compound calls was increased with the 0.50 mg/kg dose of Cirazoline compared to vehicle ($p=0.011$) and 0.125 mg/kg ($p=0.003$) (Figure 4B). There were no main effects of Cirazoline on duration of simple or complex calls ($p>0.05$; Supplemental Table 3, 4).

3.1.4 Bandwidth—There were no main effects of Cirazoline on bandwidth of simple, complex, or compound calls ($p>0.05$; Supplemental Tables 3–5).

3.1.5 Intensity—There were no main effects of Cirazoline on intensity for simple, complex, or compound calls ($p>0.05$; Supplemental Tables 3–5).

3.1.6 Peak Frequency—There were no main effects of drug treatment on peak frequency of simple, complex or compound calls ($p<0.05$; Supplemental Tables 3–5).

3.2 Prazosin

3.2.1 Locomotor and Latency to Mount—There was not a main effect of Prazosin on latency to mount ($p>0.05$). There were also no main effects of drug treatment on the number of cage crossings, number of rears, time spent eating, time spent grooming or any of the locomotor variables ($p>0.05$) (See Supplemental Table 1).

3.2.2 General Vocalization Properties—There was a main effect of drug treatment on call rate [$F(3,27)=15.26$, $p<0.001$]. Call rate was significantly decreased with 0.1 mg/kg ($p=0.048$), 0.3 mg/kg ($p<0.001$), and 1.0 mg/kg ($p<0.001$) of Prazosin compared to vehicle. Additionally, 0.3 mg/kg and 1.0 mg/kg also demonstrated significantly reduced call rate compared to 0.1 mg/kg ($p=0.006$ and $p<0.001$, respectively) (Figure 3). There was a main effect of drug treatment on the standard deviation of intensity [$F(3,26)=5.6$, $p=0.004$]. Standard deviation of intensity was significantly reduced with 1.0 mg/kg of Prazosin compared to vehicle ($p<0.001$) and 0.3 mg/kg ($p=0.035$) and with 0.1 mg/kg compared to vehicle ($p=0.034$) (Supplemental Table 2).

There was a main effect of drug treatment on the percent of compound [$F(3,27)=3.79$, $p<0.022$] and frequency modulated [$F(3,27)=4.86$, $p=0.008$] calls. The percent of compound calls was significantly reduced with the 0.3 mg/kg ($p=0.005$) and 1.0 mg/kg ($p=0.022$) doses compared to vehicle treatment (data not shown). The percent of frequency modulated calls was significantly reduced with the 0.1 mg/kg ($p=0.013$), 0.3 mg/kg ($p<0.001$), and 1.0 mg/kg ($p=0.049$) doses compared to vehicle treatment. There was not a main effect of drug treatment on latency to call or any other call types ($p>0.05$).

3.2.3 Duration—There were no main effects of drug treatment on duration for simple, complex, or compound calls ($p>0.05$; Supplemental Tables 3–5).

3.2.4 Bandwidth—There was a main effect of drug treatment on bandwidth of simple calls [$F(3,26)=3.76$, $p=0.023$]. Bandwidth of simple calls was reduced with 1.0 mg/kg of Prazosin compared to vehicle ($p=0.003$) (Figure 5A). There was no effect of Prazosin on bandwidth of complex or compound calls ($p>0.05$; Supplemental Table 4, 5).

3.2.5 Intensity—There was a main effect of drug treatment on intensity of simple calls [$F(3,26)=6.37$, $p=0.002$]. Intensity of simple calls was reduced with the 0.1 mg/kg ($p=0.027$), 0.3 mg/kg ($p=0.005$), and 1.0 mg/kg ($p<0.001$) doses of Prazosin compared to vehicle (Figure 7A).

There was also a main effect of drug treatment on intensity of complex calls [$F(3,27)=12.14$, $p<0.001$]. Intensity of complex calls was reduced with the 0.1 mg/kg ($p=0.004$), 0.3 mg/kg ($p=0.005$), and 1.0 mg/kg ($p<0.001$) doses of Prazosin compared to vehicle and the 1.0 mg/kg dose was significantly reduced compared to the 0.1 mg/kg ($p=0.007$) and 0.03 mg/kg ($p=0.006$) doses (Figure 7B).

There was not an effect of Prazosin on intensity of compound calls ($p>0.05$; Supplemental Table 5).

3.2.6 Peak Frequency—There was no effect of Prazosin on peak frequency of simple, complex, or compound calls ($p>0.05$; Supplemental Tables 3–5).

3.3 Clonidine

3.3.1 Locomotor and Latency to Mount—There was no effect of Clonidine on latency to mount ($p>0.05$). There was a main effect of Clonidine on the total number of cage

crossings [$F(3,26)=9.39$, $p<0.001$] and rears [$F(3,26)=16.55$, $p<0.001$]. The number of cage crossings was significantly reduced with 0.1 mg/kg Clonidine compared to vehicle ($p<0.001$), 0.01 mg/kg ($p<0.001$), and 0.02 mg/kg ($p=0.007$) (Figure 8A). The number of rears was significantly reduced with 0.1 mg/kg Clonidine compared to vehicle ($p<0.001$), 0.01 mg/kg ($p<0.001$), and 0.02 mg/kg ($p=0.003$) and with 0.02 mg/kg compared to vehicle ($p=0.003$) (Figure 8B). There was a main effect of drug treatment on time spent grooming [$F(3,26)=13.64$, $p<0.001$] and eating [$F(3,26)=6.56$, $p=0.002$]. Time spent grooming was significantly reduced with 0.01 mg/kg ($p=0.004$), 0.02 mg/kg ($p<0.001$), and 0.1 mg/kg ($p<0.001$) compared to vehicle and with 0.1 mg/kg compared to 0.01 mg/kg ($p=0.006$) (Figure 8C). Time spent eating was significantly reduced with 0.1 mg/kg of Clonidine compared to vehicle ($p=0.015$), 0.01 mg/kg ($p=0.007$), and 0.02 mg/kg ($p<0.001$; (Figure 8D)). See Supplemental Table 1.

3.3.2 General Vocalization Properties—There was a main effect of drug treatment on latency to call [$F(3,26)=5.02$, $p=0.007$]. Rats commenced calling significantly later following the 0.1 mg/kg ($p<0.001$) and 0.02 mg/kg ($p=0.023$) doses of Clonidine compared to vehicle. Latency to call was also significantly increased with the 0.1 mg/kg dose compared to the 0.01 mg/kg dose ($p=0.035$). There was a main effect of drug treatment on call rate [$F(3,25)=48.221$, $p<0.001$]. Clonidine dose-dependently decreased call rate ($p<0.001$ for all comparisons except 0.01 mg/kg compared to 0.02 mg/kg was $p=0.004$; Figure 3). See Supplemental Table 2.

There was a main effect of drug treatment on the percent of frequency modulated [$F(3,25)=9.572$, $p<0.001$] and ramp calls [$F(3,25)=5.97$, $p=0.003$]. Rats produced fewer frequency modulated and ramp calls at the highest dose (0.1 mg/kg) compared to vehicle ($p<0.001$; $p=0.002$, respectively), 0.01 mg/kg ($p=0.001$; $p=0.023$, respectively), and 0.02 mg/kg ($p=0.002$; $p<0.001$, respectively). In addition, there was main effect of drug treatment on the percent of harmonic [$F(3,25)=4.0$, $p=0.019$] and short calls [$F(3,25)=3.07$, $p=0.046$]. Harmonic and short calls were abolished at the highest dose. This was significantly different from vehicle ($p=0.008$) and 0.01 mg/kg ($p=0.011$) for harmonic calls and significantly different from vehicle ($p=0.011$) and 0.02 mg/kg ($p=0.022$) for short calls. There were no other main effects of drug treatment on call type ($p>0.05$).

3.3.3 Duration—There was a main effect of drug treatment on duration of compound calls [$F(3,19)=3.18$, $p=0.048$]. Duration was increased with the highest dose of Clonidine (0.1 mg/kg) compared to vehicle ($p=0.01$), 0.01 mg/kg ($p=0.011$), and 0.02 mg/kg ($p=0.033$) (Figure 4B). There were no main effects of drug treatment on duration of simple or complex calls ($p>0.05$; Supplemental Table 3, 4).

3.3.4 Bandwidth—There was a main effect of drug treatment on bandwidth of complex calls [$F(3,21)=12.13$, $p<0.001$]. Bandwidth of complex calls was reduced in the 0.02 mg/kg and 0.1 mg/kg doses of Clonidine compared to vehicle ($p=0.001$ and $p<0.001$, respectively) and 0.01 mg/kg ($p<0.001$ and $p=0.003$, respectively) (Figure 5B).

There was a main effect of drug treatment on bandwidth of compound calls [$F(3,19)=7.37$, $p=0.002$]. Bandwidth of compound calls was reduced in the 0.01 mg/kg ($p=0.018$), 0.02

mg/kg ($p=0.001$), and 0.1 mg/kg ($p<0.001$) doses of Clonidine compared to vehicle (Figure 5C). There was no effect of Clonidine on bandwidth of simple calls ($p>0.05$; Supplemental Table 3).

3.3.5 Intensity—There was a main effect of drug treatment on intensity of simple calls [$F(3,23)=12.19$, $p<0.001$]. Intensity of simple calls was significantly reduced (quieter) with the 0.1 mg/kg dose compared to vehicle ($p<0.001$) and 0.01 mg/kg ($p<0.001$) but increased (louder) compared to the 0.02 mg/kg ($p=0.014$). The 0.02 mg/kg dose was significantly reduced (quieter) compared to vehicle ($p=0.004$) and the 0.01 mg/kg dose ($p=0.046$) (Figure 7A).

There was a main effect of drug treatment on intensity of complex calls [$F(3,21)=4.34$, $p=0.016$]. Intensity of complex calls was significantly reduced with the 0.02 mg/kg dose compared to vehicle ($p=0.002$) and 0.01 mg/kg ($p=0.029$) (Figure 7B).

There was no effect of Clonidine on intensity of compound calls ($p>0.05$; Supplemental Table 4).

3.3.6 Peak Frequency—There was a main effect of drug treatment on peak frequency of simple calls [$F(3,23)=4.6$, $p=0.012$]. Peak frequency of simple calls was significantly reduced with the 0.1 mg/kg dose compared to vehicle ($p=0.002$), 0.01 mg/kg ($p=0.006$), and 0.02 mg/kg ($p=0.021$) (Figure 6A).

There was a main effect of drug treatment on peak frequency of complex calls [$F(3,21)=4.68$, $p=0.012$]. Peak frequency of complex calls was significantly reduced with the 0.1 mg/kg dose compared to vehicle ($p=0.003$), 0.01 mg/kg ($p=0.008$), and 0.02 mg/kg ($p=0.003$) (Figure 6B).

There was a main effect of drug treatment on peak frequency of compound calls [$F(3,19)=4.08$, $p=0.021$]. Peak frequency of compound calls was significantly reduced with the 0.1 mg/kg dose compared to vehicle ($p<0.001$), 0.01 mg/kg ($p=0.004$), and 0.02 mg/kg ($p<0.001$) (Figure 6C).

3.4 Atipamezole

3.4.1 Locomotor and Latency to Mount—There were no main effects of drug treatment on the number of cage crossings, number of rears, time spent eating, or time spent grooming ($p>0.05$ for all). There was not an effect of Atipamezole on latency to mount ($p>0.05$). See Supplemental Table 1.

3.4.2 General Vocalization Properties—There was a main effect of drug treatment on the percent of step calls [$F(3,26)=4.18$, $p=0.015$]. Rats produced significantly more step calls with 0.3 mg/kg ($p=0.028$) and 1.0 mg/kg ($p=0.003$) of Atipamezole compared to vehicle and significantly more with 1.0 mg/kg compared to 0.1 mg/kg ($p=0.035$). Atipamezole did not have any other significant effects on call profile ($p>0.05$). There were no main effects of Atipamezole on latency to call, call rate, or standard deviation of intensity ($p>0.05$).

3.4.3 Duration—There were no main effects of drug treatment on duration of simple, complex, or compound calls ($p>0.05$; Supplemental Tables 3–5).

3.4.4 Bandwidth—There were no effects of drug treatment on bandwidth of simple, complex, or compound calls ($p>0.05$; Supplemental Tables 3–5).

3.4.5 Intensity—There was a main effect of drug treatment on intensity of simple calls [$F(3,26)=3.7$, $p=0.024$]. Intensity was increased with the highest dose of Atipamezole, 1.0 mg/kg ($p=0.008$), and middle dose, 0.3 mg/kg ($p=0.006$), compared to the lowest dose, 0.1 mg/kg (Figure 7A). There was no effect of Atipamezole on the intensity of complex or compound calls ($p>0.05$; Supplemental Table 4, 5).

3.4.6 Peak Frequency—There were no main effects of drug treatment on peak frequency of simple, complex or compound calls ($p>0.05$ for all; Supplemental Tables 3–5).

3.5 Propranolol

3.5.1 Locomotor and Latency to Mount—There was no effect of Propranolol on latency to mount ($p>0.05$) or the number of cage crossings, number of rears, time spent eating, or time spent grooming ($p>0.05$). See Supplemental Table 1.

3.5.2 General Vocalization Properties—There was a main effect of drug treatment on the call rate [$F(3,27)=3.00$, $p=0.048$]. Call rate was significantly increased with all the doses compared to vehicle ($p=0.024$, $p=0.035$, and $p=0.013$ for 0.3, 1.0, and 3.0 mg/kg, respectively). There was no effect of Propranolol on latency to call or standard deviation of intensity ($p>0.05$). See Supplemental Table 2.

3.5.3 Duration—There was a main effect of drug treatment on the duration of simple calls [$F(3,27)=3.52$, $p=0.028$]. Duration was increased with the highest dose, 3.0 mg/kg Propranolol compared to vehicle (Figure 4A). There were no main effects of drug treatment on duration of complex or compound calls ($p>0.05$; Supplemental Table 4, 5).

3.5.4 Bandwidth—There were no main effects of drug treatment on bandwidth of simple, complex or compound calls ($p>0.05$; Supplemental Tables 3–5).

3.5.5 Intensity—There was a main effect of Propranolol on the intensity of complex calls [$F(3,27)=3.540$, $p=0.028$]. Intensity of complex calls was significantly increased with the 1.0 mg/kg and 3.0 mg/kg doses of Propranolol ($p=0.035$ and $p=0.004$, respectively) compared to vehicle (Figure 7B). While there was no main effects of drug treatment on intensity of simple or compound calls ($p>0.05$), there was a general trend towards increasing intensity with increasing dose.

3.5.6 Peak Frequency—There was a main effect of drug treatment on the peak frequency of simple [$F(3,27)=3.67$, $p=0.025$] and complex [$F(3,27)=3.66$, $p=0.025$] calls. There was a significant decrease in peak frequency of simple calls at 1.0 mg/kg ($p=0.036$) and 3.0 mg/kg ($p=0.003$) (Figure 6A) compared to vehicle. Similarly, there was a significant decrease in

peak frequency of complex calls at 1.0 mg/kg ($p=0.007$) and 3.0 mg/kg ($p=0.012$) (Figure 6B).

4. Discussion

We hypothesized that pharmacological manipulation of the noradrenergic system would have a direct effect on rat vocalizations and the results of the present study indicate that specific acoustic properties of USV can be modulated by noradrenergic mechanisms. Specifically, this study found that an α_2 receptor agonist (Clonidine) or α_1 receptor antagonist (Prazosin) decreased call rate, and a β receptor antagonist (Propranolol) increased call profile, while an α_1 receptor agonist or an α_2 receptor antagonist (Cirazoline and Atipamezole, respectively) had no effect on call rate. This work establishes that modulation of NE ARs results in changes in general USV features such as call rate and profile as previously observed (Wright, Dobosiewicz and Clarke, 2012), and NE receptor subtypes contribute differentially to pertinent aspects of USV such as intensity (loudness), bandwidth, duration, and peak frequency.

4.1 Adrenoceptors differentially modulated ultrasonic calls

Modulation of specific noradrenergic receptor subtypes (α_1 -, α_2 - and β -AR) with agonists and antagonists results in differential effects on call rate as well as the types of calls produced (call profile). We found that Cirazoline resulted in a dose-dependent decrease in the number of flat calls without affecting call rate. In addition, we observed dose-dependent effects of Cirazoline on the average duration of compound calls (increased). Prazosin, on the other hand, dose-dependently decreased call rate, significantly reduced the percent of compound and frequency modulated calls, and decreased bandwidth of compound calls. Intensity was also reduced for simple and complex calls. These findings indicate that the α_1 -AR is important for mediating not only the number of calls produced (Wright, Dobosiewicz and Clarke, 2012), but may also contribute to the control of bandwidth, duration, and intensity.

We found that Atipamezole resulted in dose dependent increases in the number of step calls and average intensity of simple calls. In contrast, Clonidine, resulted in increased latency to call, dose dependent decreases in call rate, the percent of frequency modulated, ramp, harmonic, and short calls. Duration of compound calls was increased, while bandwidth was dose-dependently reduced for compound and complex calls. Intensity was reduced for simple and complex calls, and peak frequency was reduced for all calls. Given that Clonidine has been shown to have sedative effects (Drew, Gower and Marriott, 1979), it is possible that the broad locomotor (see below) and USV effects observed here are related to level of arousal. Propranolol was the only drug that dose-dependently increased call rate and intensity of complex calls. We also saw decreases in peak frequency of simple and complex calls, and increased duration of simple calls with Propranolol. Again, these findings suggest that NE-ARs, specifically the β -AR and α_2 -AR, may be important for mediating intensity, or loudness, of USV. In general, these findings indicate that specific noradrenergic mechanisms contribute to vocal control, but the relationship is not linear.

In the current study, we found that drugs that antagonize α_1 -AR (Prazosin) or reduced NE release (by agonizing presumptive α_2 autoreceptors (Clonidine)) resulted in significant reductions in multiple USV parameters. Clonidine resulted in decreased complexity of call profile (frequency modulated, harmonic, ramp, and short calls were all reduced and most calls were flat), dose-dependently reduced call rate, and reduced intensity, bandwidth, and peak frequency. Similarly, Prazosin altered call profile (reduced compound and frequency-modulated calls) and reduced call rate, bandwidth, and intensity. This trend was not universal; however, as the β receptor antagonist Propranolol resulted in increased call rate and intensity. Despite this exception, the results of this study indicate that decreasing NE transmission at the α_1 -AR (Prazosin) and α_2 -AR (Clonidine) results in quantitatively negative effects on call rate, intensity, peak frequency, and bandwidth. Although increasing NE transmission or stimulating α_1 NE receptors directly had relatively few effects, these effects were uniform in nature. Atipamezole resulted in increased intensity (loudness) at the two highest doses, as well as more step-calls. Similarly, at the highest dose, Cirazoline resulted in increased duration at and an increase in the number of flat calls. No decreases in any acoustic parameters were observed following Atipamezole or Cirazoline.

4.2 Locomotor activity

In addition to vocalizations, we also evaluated locomotor activity as a measure of gross sensorimotor function. We found that Prazosin, Atipamezole, and Propranolol did not have any effect on the number of cage crossings, number of rears, times spent eating, or time spent grooming, yet these compounds did change USV. The number of rears was dose-dependently decreased following Cirazoline, but the only effect on USV was on duration of compound calls. Clonidine dose-dependently decreased all aspects of locomotor activity and significantly affected USV. Additionally, latency to call was only increased with Clonidine. Given that rats vocalize when moving around, we cannot rule out the possibility that the effects of Clonidine on latency to call and USV parameters were due to general sedation effects [(Drew, Gower and Marriott, 1979). However, locomotion alone does not, in and of itself result in vocalizations (Knutson, Burgdorf and Panksepp, 2002). In the present study, we found compounds that affected USV without altering locomotion. Therefore, altered locomotion in neither necessary nor sufficient to affect USV.

4.3 Current findings in relation to previous studies

The results of this study generally support previous work with amphetamine-induced rat USV and add to what is known about the contribution of noradrenergic mechanisms to vocal control. We found reductions in call rate with Clonidine and Prazosin and no changes in call rate with Atipamezole and Cirazoline. We observed alterations in USV intensity, bandwidth, and peak frequency with modulation of each receptor. Wright *et al.*, 2012 (Wright, Dobosiewicz and Clarke, 2012) reported no changes in call rate with Atipamezole without amphetamine and few changes in call profile (increase in step and short calls) with amphetamine. Our results are partially consistent with this, as we did not observe changes in call rate and the only change in call profile was a decrease in step calls with Atipamezole. Taken together, these results indicate that the α_2 -AR may contribute to the types of calls produced, but that the contribution may be dependent upon context. Specifically, call profile changes when amphetamine is used to induce USV (Wright, Dobosiewicz and Clarke, 2012)

and this differs from the call profile when a social, mating paradigm is used (current study). Given that amphetamine alone can induce changes in USV production, (Burgdorf, Knutson, Panksepp and Ikemoto, 2001; Ahrens, Ma, Maier, Duvauchelle and Schallert, 2009; Wright, Gourdon and Clarke, 2010; Brudzynski, Silkstone, Komadoski, Scullion, Duffus et al., 2011; Wright, Dobosiewicz and Clarke, 2012; Wright, Dobosiewicz and Clarke, 2013) it is possible that amphetamine affects on both dopamine and NE could account for these differences. There are some minor methodologic differences in doses but route and timing were consistent. An alternative, but not mutually exclusive, explanation is that context matters. Given the role of NE in goal directed behaviors (Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005; Chamberlain and Robbins, 2013), it would not be surprising if alterations to USV following NE modifying drugs enhanced/diminished vocal behaviors as a function of social context.

4.4 Limitations and clinical implications

The effects (or lack of effects) observed are limited to the dose ranges specifically tested in this study. In a diseased state, the effects of these drugs and doses might not be the same due to the up/down regulation of noradrenergic-ARs because of the disease process. However, this study suggests that noradrenergic mechanisms are involved in the modulation of pertinent vocal parameters and in some cases Propranolol result in potentially desirable effects (increased call rate and intensity).

Rat USVs are currently being used at the preclinical level to study the effects elicited by different classes of drugs, and to study the neurobiological mechanisms of these effects. Interestingly, we found that Propranolol increased intensity, or loudness, of USV. Reduced loudness is a hallmark feature of dysphonia in humans with Parkinson disease (Darley, Aronson and Brown, 1968; Darley, Aronson and Brown, 1975; Ho, Iannsek, Marigliani, Bradshaw and Gates, 1998; Plowman-Prine, Okun, Sapienza, Shrivastav, Fernandez et al., 2009) and likewise, is almost universally affected in rodent models of Parkinson disease (Ciucci, Ma, Fox, Kane, Ramig et al., 2007; Ciucci, Ahrens, Ma, Kane, Windham et al., 2009; Gombash, Manfredsson, Kemp, Kuhn, Fleming et al., 2013; Grant, Richter, Miller, White, Fox et al., 2014; Grant, Barnett, Doll, Levenson and Ciucci, 2015; Grant, Kelm-Nelson, Hilby, Blue, Paul Rajamanickam et al., 2015). We also found increased duration of complex calls with Cirazoline and Atipamezole. While the mechanisms underlying this increase are not clear, some potential explanations include increased arousal and/or attention (Berridge, Arnsten and Foote, 1993; Berridge and Waterhouse, 2003) and increased sexual motivation. (Pertovaara, Haapalinna, Sirvio and Virtanen, 2005; Viitamaa, Haapalinna and Agmo, 2006) However, while Atipamezole has been shown to increase sexual activity and NE activity in regions important for mediating sexual behavior, (Pertovaara, Haapalinna, Sirvio and Virtanen, 2005; Viitamaa, Haapalinna and Agmo, 2006) we did not observe differences in latency to mount for any of the drugs used in this study. Propranolol also increased call rate, which was not the case in previous work (Wright, Dobosiewicz and Clarke, 2012). This discrepancy is likely due to methodological differences in how calls are elicited, as the dose range used in Wright *et al.*, 2012 (Wright, Dobosiewicz and Clarke, 2012) included all the doses used in the current study. The findings in the present study

indicate that noradrenergic receptors (specifically the α_2 -AR and β -AR) may be viable targets for attenuating voice deficits in Parkinson disease-particularly vocal intensity.

4.5 Relevance to disease states

Parkinson disease is devastating to voice and communication function, negatively influencing social interactions and quality of life (Ho, Iannsek, Marigliani, Bradshaw and Gates, 1998; Plowman-Prine, Okun, Sapienza, Shrivastav, Fernandez et al., 2009). Voice and communication deficits do not respond to standard interventions targeting the primary disease pathology of nigrostriatal dopamine depletion (Dromey, Kumar, Lang and Lozano, 2000; Schulz and Grant, 2000; Pinto, Thobois, Costes, Le Bars, Benabid et al., 2004; Klostermann, Ehlen, Vesper, Nubel, Gross et al., 2008; Narayana, Jacks, Robin, Poizner, Zhang et al., 2009). The refractory nature of dysphonia to levodopa along with evidence that voice deficits may emerge early in the progression of Parkinson disease prior to significant nigrostriatal dopamine loss (Stewart, Winfield, Hunt, Bressman, Fahn et al., 1995; Harel, Cannizzaro and Snyder, 2004; Harel, Cannizzaro, Cohen, Reilly and Snyder, 2004; Rusz, Cmejla, Ruzickova, Klempir, Majerova et al., 2011; Rusz, Cmejla, Ruzickova and Ruzicka, 2011; Rusz, Cmejla, Ruzickova, Klempir, Majerova et al., 2013), implicate other mechanisms. These data suggest that the neuropathology underlying dysphonia could involve NE mechanisms.

In humans, NE has been implicated in the treatment of cognitive and motor dysfunction in Parkinson disease (Marien, Colpaert and Rosenquist, 2004; Rommelfanger and Weinshenker, 2007). Likewise, vocal communication deficits associated with potential NE involvement have been observed in transgenic and genetic models of Parkinson disease (Grant, Richter, Miller, White, Fox et al., 2014; Grant, Kelm-Nelson, Hilby, Blue, Paul Rajamanickam et al., 2015). For example, mice overexpressing human wild-type alpha-synuclein (aSyn; Thy1-aSyn) show alterations in call profile, intensity and duration of USV, (Grant, Richter, Miller, White, Fox et al., 2014) while *Pink1* $-/-$ rats demonstrate reduced intensity, bandwidth, and peak frequency of USV (Grant, Kelm-Nelson, Hilby, Blue, Paul Rajamanickam et al., 2015). In both the transgenic mouse and genetic rat models, vocalization deficits emerge early, independent of nigrostriatal dopamine loss, and each have neuropathological findings in the locus coeruleus, such as aSyn aggregates in the Thy1-aSyn mouse model (Fernagut, Hutson, Fleming, Tetreaut, Salcedo et al., 2007) and aSyn aggregates and reduced tyrosine hydroxylase-immunoreactivity in *Pink1* $-/-$ rat model (Grant, Kelm-Nelson, Hilby, Blue, Paul Rajamanickam et al., 2015). Reductions in tyrosine hydroxylase-immunoreactivity in the locus coeruleus of *Pink1* $-/-$ rats is significantly correlated with reductions in intensity, indicating that reductions in norepinephrine may contribute to decreased loudness in this model (Grant, Kelm-Nelson, Hilby, Blue, Paul Rajamanickam et al., 2015). The *Pink1* $-/-$ rat also shows reductions in norepinephrine protein concentrations in the locus coeruleus in conjunction with increases in catechol-O-methyltransferase mRNA, the key enzyme in norepinephrine degradation (Kelm-Nelson, Trevino and Ciucci, 2018). However, these studies merely indicate a relationship between norepinephrine and vocal control. The results from the current study provide further evidence those alterations to NE signaling may contribute to vocal deficits in disease states.

4.5 Conclusions

We observed that alterations in NE signaling (increases and decreases) impact multiple USV parameters while preserving gross locomotor behaviors, underscoring a possible dissociation of USV (axial deficits) from gross motor function in the limb (appendicular deficits). These findings validate and extend previous work demonstrating that general features such as call profile and rate change with administration of NE agonists and antagonists. In addition, drugs that act at different AR subtypes alter USV parameters in similar ways, suggesting that there might be an optimal range of noradrenergic signaling in which vocal motor control functions best. Finally, two drugs (Atipamezole and Propranolol) increased vocal loudness (intensity), indicating that NE-modulating drugs may be effective for attenuating Parkinson disease-induced voice deficits, which are currently undertreated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge John Szot for managerial support and our funding sources: T32 DC009401 (Grant), F32 D014399 (Kelm-Nelson), R21 DC016135 (Kelm-Nelson), R01DC014358 (Ciucci), UW Department of Surgery (Ciucci).

References

- 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. *Behavioural brain research*. 2009; 197(1):205. [PubMed: 18809437]
- Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci*. 2005; 28:403–450. [PubMed: 16022602]
- Barclay SR, Harding CF, Waterman SA. Central DSP-4 treatment decreases norepinephrine levels and courtship behavior in male zebra finches. *Pharmacol Biochem Behav*. 1996; 53(1):213–220. [PubMed: 8848453]
- Berridge CW, Arnsten AF, Foote SL. Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological and behavioural studies in animal models. *Psychol Med*. 1993; 23(3):557–564. [PubMed: 8234565]
- Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev Netherlands*. 2003; 42:33–84.
- Bialer M, Johannessen SI, Kupferberg HJ, Levy RH, Perucca E, Tomson T. Progress report on new antiepileptic drugs: a summary of the Seventh Eilat Conference (EILAT VII). *Epilepsy Res*. 2004; 61(1–3):1–48. [PubMed: 15570674]
- Brudzynski S. Principles of Rat Communication: Quantitative Parameters of Ultrasonic Calls in Rats. *Behavior Genetics*. 2005; 35(1):85–92. [PubMed: 15674535]
- Brudzynski SM. Handbook of Behavioral NeuroscienceElsevier; 2010Handbook of Mammalian Vocalization: An Integrative Neuroscience Approach; 19
- Brudzynski SM, Komadoski M, St Pierre J. Quinpirole-induced 50 kHz ultrasonic vocalization in the rat: role of D2 and D3 dopamine receptors. *Behav Brain Res*. 2012; 226(2):511–518. [PubMed: 22015714]
- Brudzynski SM, Silkstone M, Komadoski M, Scullion K, Duffus S, Burgdorf J, Kroes RA, Moskal JR, Panksepp J. Effects of intraaccumbens amphetamine on production of 50 kHz vocalizations in

- three lines of selectively bred Long-Evans rats. *Behav Brain Res.* 2011; 217(1):32–40. [PubMed: 20937325]
- Burgdorf J, Knutson B, Panksepp J, Ikemoto S. Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behavioral neuroscience.* 2001; 115(4):940. [PubMed: 11508733]
- Burgdorf J, Wood PL, Kroes RA, Moskal JR, Panksepp J. Neurobiology of 50-kHz ultrasonic vocalizations in rats: Electrode mapping, lesion, and pharmacology studies. *Behavioural Brain Research.* 2007; 182(2):274–283. [PubMed: 17449117]
- Castelino CB, Schmidt MF. What birdsong can teach us about the central noradrenergic system. *Journal of Chemical Neuroanatomy.* 2010; 39(2):96–111. [PubMed: 19686836]
- Chamberlain SR, Robbins TW. Noradrenergic modulation of cognition: Therapeutic implications. *J Psychopharmacol.* 2013
- Ciucci MR, Ahrens AM, Ma ST, Kane JR, Windham EB, Woodlee MT, Schallert T. Reduction of dopamine synaptic activity: Degradation of 50-kHz ultrasonic vocalization in rats. *Behavioral Neuroscience.* 2009; 123(2):328–336. [PubMed: 19331456]
- Ciucci MR, Ahrens AM, Ma ST, Kane JR, Windham EB, Woodlee MT, Schallert T. Reduction of dopamine synaptic activity: degradation of 50-kHz ultrasonic vocalization in rats. *Behavioral neuroscience.* 2009; 123(2):328. [PubMed: 19331456]
- Ciucci MR, Connor NP. Dopaminergic influence on rat tongue function and limb movement initiation. *Experimental Brain Research.* 2009; 194(4):587–596. [PubMed: 19247644]
- Ciucci MR, Ma ST, Fox C, Kane JR, Ramig LO, Schallert T. Qualitative changes in ultrasonic vocalization in rats after unilateral dopamine depletion or haloperidol: A preliminary study. *Behavioural Brain Research.* 2007; 182(2):284–289. [PubMed: 17397940]
- Ciucci MR, Ma ST, Fox C, Kane JR, Ramig LO, Schallert T. Qualitative changes in ultrasonic vocalization in rats after unilateral dopamine depletion or haloperidol: a preliminary study. *Behavioural brain research.* 2007; 182(2):284. [PubMed: 17397940]
- Conway EL, Jarrott B. Clonidine distribution in the rat: temporal relationship between tissue levels and blood pressure response. *Br J Pharmacol.* 1980; 71(2):473–478. [PubMed: 7470758]
- Costa G, Morelli M, Simola N. Involvement of Glutamate NMDA Receptors in the Acute, Long-Term, and Conditioned Effects of Amphetamine on Rat 50 kHz Ultrasonic Vocalizations. *Int J Neuropsychopharmacol.* 2015; 18(11):pyv057. [PubMed: 25991653]
- Costantini F, D'Amato RF. Ultrasonic vocalizations in mice and rats: social contexts and functions. *Acta zoologica Sinica.* 2006; 52(4):619–633.
- DarleyF, , AronsonA, , BrownJ. *Motor speech disorders* Philadelphia: Saunders; 1975
- Darley FL, Aronson AE, Brown JR. Motor speech signs in neurologic disease. *The Medical clinics of North America.* 1968; 52(4):835. [PubMed: 5742068]
- Drew GM, Gower AJ, Marriott AS. Alpha 2-adrenoceptors mediate clonidine-induced sedation in the rat. *Br J Pharmacol.* 1979; 67(1):133–141. [PubMed: 40643]
- Dromey C, Kumar R, Lang AE, Lozano AM. An investigation of the effects of subthalamic nucleus stimulation on acoustic measures of voice. *Movement disorders : official journal of the Movement Disorder Society.* 2000; 15(6):1132. [PubMed: 11104196]
- Elghozi JL, Bianchetti G, Morselli PL, Meyer P. Brain distribution of propranolol in the rat. *Eur J Pharmacol.* 1979; 55(3):319–322. [PubMed: 456429]
- Fernagut PO, Hutson CB, Fleming SM, Tetreaut NA, Salcedo J, Masliah E, Chesselet MF. Behavioral and histopathological consequences of paraquat intoxication in mice: effects of alpha-synuclein over-expression. *Synapse (New York, NY).* 2007; 61(12):991.
- Fornai F, Alessandri MG, Fascetti F, Vaglini F, Corsini GU. Clonidine suppresses 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced reductions of striatal dopamine and tyrosine hydroxylase activity in mice. *J Neurochem.* 1995; 65(2):704–709. [PubMed: 7616226]
- Gombash SE, Manfredsson FP, Kemp CJ, Kuhn NC, Fleming SM, Egan AE, Grant LM, Ciucci MR, MacKeigan JP, Sortwell CE. Morphological and behavioral impact of AAV2/5-mediated overexpression of human wildtype alpha-synuclein in the rat nigrostriatal system. *PLoS One.* 2013; 8(11):e81426. [PubMed: 24312298]

- Grant LM, Barnett DG, Doll EJ, Levenson G, Ciucci M. Relationships among rat ultrasonic vocalizations, behavioral measures of striatal dopamine loss, and striatal tyrosine hydroxylase immunoreactivity at acute and chronic time points following unilateral 6-hydroxydopamine-induced dopamine depletion. *Behav Brain Res*. 2015; 291:361–371. [PubMed: 26026785]
- Grant LM, Kelm-Nelson CA, Hilby BL, Blue KV, Paul Rajamanickam ES, Pultorak JD, Fleming SM, Ciucci MR. Evidence for early and progressive ultrasonic vocalization and oromotor deficits in a PINK1 gene knockout rat model of Parkinson's disease. *J Neurosci Res*. 2015
- Grant LM, Kelm-Nelson CK, Hilby BL, Blue KV, Rajamanickam ESP, Pultorak J, Fleming SM, Ciucci MR. Evidence for early and progressive ultrasonic vocalization and oromotor deficits in a PINK1 knockout rat model of Parkinson disease. *Journal of Neuroscience Research*. 2015; 93(11):1713–1727. [PubMed: 26234713]
- Grant LM, Richter F, Miller JE, White SA, Fox CM, Zhu C, Chesselet MF, Ciucci MR. Vocalization deficits in mice over-expressing alpha-synuclein, a model of pre-manifest Parkinson's disease. *Behav Neurosci*. 2014; 128(2):110–121. [PubMed: 24773432]
- Grant LM, Richter FR, Miller JE, White SA, Fox CM, Zhu C, Chesselet MF, Ciucci MR. Vocalization deficits in mice over-expressing alpha-synuclein, a model of pre-manifest Parkinson's disease. *Behavioral Neuroscience*. 2014; 128(2):110–121. [PubMed: 24773432]
- Hamed A, Daszczuk P, Kurska MB, Turzynska D, Sobolewska A, Lehner M, Boguszewski PM, Szyndler J. Non-parametric analysis of neurochemical effects and Arc expression in amphetamine-induced 50-kHz ultrasonic vocalization. *Behav Brain Res*. 2016; 312:174–185. [PubMed: 27288591]
- Hamed A, Szyndler J, Taracha E, Turzynska D, Sobolewska A, Lehner M, Krzascik P, Daszczuk P. kappa-opioid receptor as a key mediator in the regulation of appetitive 50-kHz ultrasonic vocalizations. *Psychopharmacology (Berl)*. 2015; 232(11):1941–1955. [PubMed: 25466704]
- Harel B, Cannizzaro M, Snyder PJ. Variability in fundamental frequency during speech in prodromal and incipient Parkinson's disease: a longitudinal case study. *Brain and cognition*. 2004; 56(1):24. [PubMed: 15380872]
- Harel BT, Cannizzaro MS, Cohen H, Reilly N, Snyder PJ. Acoustic characteristics of Parkinsonian speech: a potential biomarker of early disease progression and treatment. *Journal of Neurolinguistics*. 2004; 17(6):439–453.
- Heimovics SA, Cornil CA, Ellis JMS, Ball GF, Ritters LV. Seasonal and individual variation in singing behavior correlates with alpha 2-noradrenergic receptor density in brain regions implicated in song, sexual, and social behavior. *Neuroscience*. 2011; 182(0):133–143. [PubMed: 21397668]
- Ho AK, Ianseck R, Marigliani C, Bradshaw JL, Gates S. Speech impairment in a large sample of patients with Parkinson's disease. *Behavioural neurology*. 1998; 11(3):131. [PubMed: 11568413]
- Jaillon P. Clinical pharmacokinetics of prazosin. *Clin Pharmacokinet*. 1980; 5(4):365–376. [PubMed: 6994981]
- Jaiswal MK, Mallick BN. Prazosin modulates rapid eye movement sleep deprivation-induced changes in body temperature in rats. *J Sleep Res*. 2009; 18(3):349–356. [PubMed: 19552734]
- Johnson AM, Doll EJ, Grant LM, Ringel L, Shier JN, Ciucci MR. Targeted training of ultrasonic vocalizations in aged and Parkinsonian rats. *J Vis Exp*. 2011; (54)
- Johnson AM, Doll EJ, Grant LM, Ringel L, Shier JN, Ciucci MR. Targeted Training of Ultrasonic Vocalizations in Aged and Parkinsonian Rats. *JOVE*. 2011; (54):e2835.
- Kehoe P, Harris JC. Ontogeny of noradrenergic effects on ultrasonic vocalizations in rat pups. *Behav Neurosci*. 1989; 103(5):1099–1107. [PubMed: 2553059]
- Kelm-Nelson CA, Trevino MA, Ciucci MR. Quantitative analysis of catecholamines in the Pink1 $-/-$ rat model of early-onset Parkinson disease. *Neuroscience*. 2018 In Press.
- Klostermann F, Ehlen F, Vesper J, Nubel K, Gross M, Marzinzik F, Curio G, Sappok T. Effects of subthalamic deep brain stimulation on dysarthrophonia in Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry*. 2008; 79(5):522.
- Knutson B, Burgdorf J, Panksepp J. Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin*. 2002; 128(6):961–977. [PubMed: 12405139]
- Knutson B, Burgdorf J, Panksepp J. Ultrasonic vocalizations as indices of affective states in rats. *Psychological bulletin*. 2002; 128(6):961. [PubMed: 12405139]

- Marien MR, Colpaert FC, Rosenquist AC. Noradrenergic mechanisms in neurodegenerative diseases: a theory. *Brain research Brain research reviews*. 2004; 45(1):38. [PubMed: 15063099]
- McGinnis MY, Vakulenko M. Characterization of 50-kHz ultrasonic vocalizations in male and female rats. *Physiology & Behavior*. 2003; 80(1):81–88. [PubMed: 14568311]
- Narayana S, Jacks A, Robin DA, Poizner H, Zhang W, Franklin C, Liotti M, Vogel D, Fox PT. A noninvasive imaging approach to understanding speech changes following deep brain stimulation in Parkinson's disease. *American journal of speech-language pathology/American Speech-Language-Hearing Association*. 2009; 18(2):146.
- Pertovaara A, Haapalinna A, Sirvio J, Virtanen R. Pharmacological properties, central nervous system effects, and potential therapeutic applications of atipamezole, a selective α_2 -adrenoceptor antagonist. *CNS Drug Rev*. 2005; 11(3):273–288. [PubMed: 16389294]
- Pinto S, Thobois S, Costes N, Le Bars D, Benabid AL, Broussolle E, Pollak P, Gentil M. Subthalamic nucleus stimulation and dysarthria in Parkinson's disease: a PET study. *Brain : a journal of neurology*. 2004; 127(Pt 3):602. [PubMed: 14736753]
- Plowman-Prine EK, Okun MS, Sapienza CM, Shrivastav R, Fernandez HH, Foote KD, Ellis C, Rodriguez AD, Burkhead LM, Rosenbek JC. Perceptual characteristics of Parkinsonian speech: A comparison of the pharmacological effects of levodopa across speech and non-speech motor systems. *Neurorehabilitation*. 2009; 24(2):131–144. [PubMed: 19339752]
- Portfors CV. Types and Functions of Ultrasonic Vocalizations in Laboratory Rats and Mice. *American Association for Laboratory Animal Science*. 2007; 46(1):28–34.
- Riede T. Stereotypic laryngeal and respiratory motor patterns generate different call types in rat ultrasound vocalization. *J Exp Zool A Ecol Genet Physiol*. 2013; 319(4):213–224. [PubMed: 23423862]
- Riede T. Rat ultrasonic vocalization shows features of a modular behavior. *J Neurosci*. 2014; 34(20):6874–6878. [PubMed: 24828641]
- Ringel LE, Basken JN, Grant LM, Ciucci MR. Dopamine D1 and D2 receptor antagonism effects on rat ultrasonic vocalizations. *Behav Brain Res*. 2013; 252:252–259. [PubMed: 23764460]
- Riters LV, Pawlisch BA. Evidence that norepinephrine influences responses to male courtship song and activity within song control regions and the ventromedial nucleus of the hypothalamus in female European starlings. *Brain Research*. 2007; 1149:127–140. [PubMed: 17379191]
- Rohrer DK, Kobilka BK. Insights from in vivo modification of adrenergic receptor gene expression. *Annu Rev Pharmacol Toxicol*. 1998; 38:351–373. [PubMed: 9597159]
- Rommelfanger KS, Weinshenker D. Norepinephrine: The redheaded stepchild of Parkinson's disease. *Biochemical pharmacology*. 2007; 74(2):177. [PubMed: 17416354]
- Rusz J, Cmejla R, Ruzickova H, Klempir J, Majerova V, Picmausova J, Roth J, Ruzicka E. Acoustic assessment of voice and speech disorders in Parkinson's disease through quick vocal test. *Movement disorders : official journal of the Movement Disorder Society*. 2011; 26(10):1951. [PubMed: 21484873]
- Rusz J, Cmejla R, Ruzickova H, Klempir J, Majerova V, Picmausova J, Roth J, Ruzicka E. Evaluation of speech impairment in early stages of Parkinson's disease: a prospective study with the role of pharmacotherapy. *J Neural Transm*. 2013; 120(2):319–329. [PubMed: 22772465]
- Rusz J, Cmejla R, Ruzickova H, Ruzicka E. Quantitative acoustic measurements for characterization of speech and voice disorders in early untreated Parkinson's disease. *The Journal of the Acoustical Society of America*. 2011; 129(1):350. [PubMed: 21303016]
- Scardocho T, Clarke PB. Inhibition of 50-kHz ultrasonic vocalizations by dopamine receptor subtype-selective agonists and antagonists in adult rats. *Psychopharmacology (Berl)*. 2013; 226(3):589–600. [PubMed: 23192317]
- Schulz GM, Grant MK. Effects of speech therapy and pharmacologic and surgical treatments on voice and speech in Parkinson's disease: a review of the literature. *Journal of communication disorders*. 2000; 33(1):59. [PubMed: 10665513]
- Simola N, Costa G, Morelli M. Activation of adenosine A(2)A receptors suppresses the emission of pro-social and drug-stimulated 50-kHz ultrasonic vocalizations in rats: possible relevance to reward and motivation. *Psychopharmacology (Berl)*. 2016; 233(3):507–519. [PubMed: 26564233]

- Smeets WJ, Gonzalez A. Catecholamine systems in the brain of vertebrates: new perspectives through a comparative approach. *Brain Res Brain Res Rev.* 2000; 33(2–3):308–379. [PubMed: 11011071]
- Stewart C, Winfield L, Hunt A, Bressman SB, Fahn S, Blitzer A, Brin MF. Speech dysfunction in early Parkinson's disease. *Mov Disord.* 1995; 10(5):562–565. [PubMed: 8552106]
- Viitamaa T, Haapalinna A, Agmo A. The adrenergic alpha2 receptor and sexual incentive motivation in male rats. *Pharmacol Biochem Behav.* 2006; 83(3):360–369. [PubMed: 16574206]
- Wöhr M, Houx B, Schwarting RKW, Spruijt B. Effects of experience and context on 50-kHz vocalizations in rats. *Physiology & Behavior.* 2008; 93(4–5):766–776. [PubMed: 18191963]
- Wohr M, Rippberger H, Schwarting RK, van Gaalen MM. Critical involvement of 5-HT2C receptor function in amphetamine-induced 50-kHz ultrasonic vocalizations in rats. *Psychopharmacology (Berl).* 2015; 232(10):1817–1829. [PubMed: 25417553]
- Wohr M, van Gaalen MM, Schwarting RK. Affective communication in rodents: serotonin and its modulating role in ultrasonic vocalizations. *Behav Pharmacol.* 2015; 26(6):506–521. [PubMed: 26221830]
- Wright J, Dobosiewicz MS, Clarke PS. The role of dopaminergic transmission through D1-like and D2-like receptors in amphetamine-induced rat ultrasonic vocalizations. *Psychopharmacology.* 2013; 225(4):853–868. [PubMed: 23052567]
- Wright JM, Dobosiewicz MR, Clarke PB. alpha- and beta-Adrenergic receptors differentially modulate the emission of spontaneous and amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats. *Neuropsychopharmacology.* 2012; 37(3):808–821. [PubMed: 22030713]
- Wright JM, Dobosiewicz MR, Clarke PB. The role of dopaminergic transmission through D1-like and D2-like receptors in amphetamine-induced rat ultrasonic vocalizations. *Psychopharmacology.* 2013; 225(4):853. [PubMed: 23052567]
- Wright JM, Dobosiewicz MRS, Clarke PBS. [alpha]- and [beta]-Adrenergic Receptors Differentially Modulate the Emission of Spontaneous and Amphetamine-Induced 50-kHz Ultrasonic Vocalizations in Adult Rats. *Neuropsychopharmacology.* 2012; 37(3):808–821. [PubMed: 22030713]
- 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.* 2010; 211(1):1. [PubMed: 20443111]

Research Highlights

- Suppression of noradrenergic signaling decreased call rate, intensity, and bandwidth.
- Increases in noradrenergic transmission showed a gain in call rate, duration, and intensity.
- Alpha-2 receptor agonism dose-dependently reduces limb-motor activity.

Drug, target, effect		Latency to Call			SD Intensity			Call Rate			Duration			Intensity			Bandwidth			Peak Frequency		
		L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H
Cirazoline α_1 - AR agonist	$\wedge$																					
Prazosin α_1 - AR antagonist	V																					
Clonidine α_2 - AR agonist	V																					
Atipamezole α_2 - AR antagonist	$\wedge$																					
Propranolol β - AR antagonist	V																					

Figure 1. Modulation of noradrenergic receptors directly influences rat ultrasonic vocalizations
 Alterations following the administration of each drug as compared to saline. Smallest cells within each column designate low (L), medium (M), and high (H) dose for each respective drug. Solid black indicates a decrease at a particular dose, diagonal lines indicate an increase, and blank (white) indicates no change. AR: adrenoceptor, V “Decreases” norepinephrine transmission, $\wedge$ “Increases” norepinephrine transmission.

Figure 2. Ultrasonic vocalization spectrograms

Representative spectrograms of each of the nine call types. Calls are subdivided by three main broad categories ((A) simple, (B) complex, and (C) compound). The y-axis is kHz and the x-axis is time (sec), scale bar is in panel C. Each red line indicates the upper frequency, peak frequency, and lower frequency of the call, respectively. Calls are from rats under vehicle conditions.

Figure 3. Modulation of adrenergic receptors influences ultrasonic call rate

Bar graphs illustrating average call rate (calls per second (sec)) $\pm$ standard error of the mean (SEM) for vehicle (white bar) and three doses of drug (low dose (light gray bar), medium dose (dark gray bar), and high dose (black bar)). Call rate was significantly decreased following each dose of Clonidine (α_2 agonist) and Prazosin (α_1 antagonist), and increased following administration of Propranolol (β antagonist). Within each respective drug, bars and asterisks indicate significant differences resulting from planned post hoc analyses (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 4. Increases in USV duration

Bar graphs showing the average duration (seconds (sec)) $\pm$ standard error of the mean (SEM) for vehicle and three doses of drug (low dose (light gray bar), medium dose (dark gray bar), and high dose (black bar)). (A) Duration of simple calls was increased at the highest dose of Propranolol (β antagonist). (B) Duration of compound calls was increased with the high dose and medium dose of Cirazoline (α_1 agonist) compared to vehicle. Duration of compound calls was significantly increased with the high dose of Clonidine (α_2 agonist) compared to vehicle, low dose, and medium dose. Within each respective drug, bars and asterisks indicate significant differences resulting from planned post hoc analyses (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 5. Variations in USV bandwidth with noradrenergic receptor modulation

Bar graphs showing the average bandwidth (Hertz (Hz)) $\pm$ standard error of the mean (SEM) for vehicle and three doses of drug (low dose (light gray bar), medium dose (dark gray bar), and high dose (black bar)). (A) Bandwidth of simple calls was significantly reduced with the high dose of Prazosin (α_1 antagonist). (B) Bandwidth of complex calls was reduced at the two highest doses of Clonidine (α_2 agonist). (C) Clonidine significantly decreased the bandwidth of compound calls at all doses. Within each respective drug, bars and asterisks indicate significant differences resulting from planned post hoc analyses (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 6. Noradrenergic pharmacology reduces peak frequency

Bar graphs showing the average peak frequency (Hertz (Hz)) $\pm$ standard error of the mean (SEM) for vehicle and three doses of drug (low dose (light gray bar), medium dose (dark gray bar), and high dose (black bar)). (A) Peak frequency of simple calls was significantly reduced at the highest dose of Clonidine (α_2 agonist). Peak frequency of simple calls was significantly reduced at the medium and high doses of Propranolol (β antagonist) compared with vehicle. (B) Peak frequency of complex calls was significantly reduced at the highest dose of Clonidine and with the medium and high dose of Propranolol. (C) Dose dependent decreases of peak frequency of compound calls with Clonidine. Within each respective drug, bars and asterisks indicate significant differences resulting from planned post hoc analyses (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 7. Ultrasonic call intensity (loudness) is modulated with noradrenergic pharmacological manipulation

Bar graphs showing the average intensity (decibels (dB)) $\pm$ standard error of the mean (SEM) for vehicle and three doses of drug (low dose (light gray bar), medium dose (dark gray bar), and high dose (black bar)). (A) Intensity of simple calls. Clonidine (α_1 agonist) and Prazosin (α_1 antagonist) significantly reduced call intensity. Atipamezole (α_2 antagonist) increased call intensity at the medium and highest doses (B) Intensity of complex calls. Clonidine and Prazosin significantly reduced intensity. Propranolol (β antagonist) significantly increased intensity at the highest and medium doses compared to vehicle. Within each respective drug, bars and asterisks indicate significant differences resulting from planned post hoc analyses (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure 8. Clonidine significantly reduces motor, grooming, and feeding activity

Bar graphs showing locomotor activity $\pm$ standard error of the mean (SEM) for vehicle and three doses of Clonidine (low dose (light gray bar), medium dose (dark gray bar), and high dose (black bar)). (A) Total number of cage crossings was significantly reduced at all dose levels compared to vehicle. (B) Total number of rears was significantly reduced at all dose levels. (C) Total time (seconds (sec)) spent grooming was significantly reduced at all dose levels compared to vehicle. (D) Total time (sec) spent eating was significantly decreased at the highest dose. Bars and asterisks indicate significant differences resulting from planned post hoc analyses (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Table 1

Overview of Drug Conditions.

Group	Drug Name	Receptor Target	Doses (route)	Time Before Testing
1	Cirazoline	α_1 - AR agonist	0, 0.125, 0.25, 0.50 mg/kg (ip)	20 min
2	Prozasin	α_1 - AR antagonist	0, 0.1, 0.3, 1.0 mg/kg (ip)	30 min
3	Clonidine	α_2 - AR agonist	0, 0.01, 0.02, 0.1 mg/kg (ip)	20 min
4	Atipamezole	α_2 - AR antagonist	0, 0.1, 0.3, 1.0 mg/kg (ip)	30 min
5	Propranolol	β -AR antagonist	0, 0.3, 1.0, 3.0 mg/kg (ip)	20 min

AR=adrenoceptor ip=intraperitoneal, min=minutes, mg/kg= milligrams/kilogram.

Table 2

Call Classification.

	Call Type	Description
Simple	Flat	Flat, unmodulated with not more than a 0.2 kHz/second rate of frequency change
	Step	Two adjoining frequencies with a jump (or step) between them
	Short	Very brief, unmodulated call
	Ramp	Slow change in frequency of at least 0.2 kHz/second
Complex	Trill	Rapid frequency modulations
	Harmonic	Very intense flat call that breaks into the upper and lower harmonic frequencies
	Frequency Modulated	Call consisting of at least 3 frequency changes.
Compound	Compound	Two or more calls combined. (ex, Flat+Trill, Ramp+Trill)

Descriptions of each of the 9 call types. Broad call categories used for detailed analysis of vocalizations (Simple, Complex, and Compound) indicated in the left column.