Published in final edited form as:

Behav Brain Res. 2013 September 1; 252: 252–259. doi:10.1016/j.bbr.2013.06.006.

Dopamine D1 and D2 receptor antagonism effects on rat ultrasonic vocalizations

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Abstract

Voice disorders manifest in the early stages of Parkinson disease (PD), suggesting the vulnerability of the laryngeal sensorimotor system to mild alterations in dopamine signaling. Previous research has demonstrated that manipulations of central dopamine result in acoustic changes in rat ultrasonic vocalization (USV) and selective manipulation of receptor subtypes results in dose dependent changes in call rate and complexity. However, no study has specifically focused on the influence of dopamine receptor subtypes on acoustic features of USV production. This study examined the influence of D₁ and D₂ receptor subtypes on voluntary laryngeal sensorimotor control (USV) and gross whole-body involvement. Rat USV acoustics and catalepsy descent time were analyzed following the administration of selective D₁ and D₂ receptor antagonists in isolation and in combination, and a vehicle control. Results support the hypothesis that degradations of the acoustic signal would be most severe following combined receptor antagonism (D₁ + D₂) compared with D₁ or D₂ receptor antagonism alone, and the vehicle (saline) condition. In addition, results indicate that selective D₁ receptor antagonism alters acoustic parameters to a greater extent than D₂ receptor antagonism. Thus, dopamine receptor subtypes appear to influence acoustic parameters to different degrees. Catalepsy descent time was longest following combined dopamine receptor antagonism but was also significantly increased with selective D₁ or D₂ antagonism. Together, these results support the potentially different contributions receptor subtypes play in cranial and limb sensorimotor control.

Keywords

Dopamine; Receptor; Antagonism; Ultrasonic vocalization; Sensorimotor control; Rat

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1. Introduction

Parkinson disease (PD) is a complex neurodegenerative condition characterized by sensorimotor dysfunction that has been largely attributed to degeneration of the nigrostriatal pathway and severe dopamine depletion [1-3]. In contrast to the wealth of research and literature devoted to examining motor disturbances in the extremities, only recently has increasing attention been dedicated to exploring cranial sensorimotor deficits, such as voice and swallowing disorders [4-7]. Approximately 90% of individuals with PD have a speech or voice disorder that negatively affects activities of daily living and quality of life [7, 8]. The high prevalence of communication deficits is not surprising given that the pathology adversely affects all major systems governing speech motor control including respiration, phonation and articulation. Importantly, there is evidence that suggests voice disorders manifest in the early stages of PD, indicating that the laryngeal sensorimotor system may be vulnerable to subtle, mild alterations in dopamine signaling [9, 10]. Characterizing the nature of this vulnerability is necessary for optimal management of voice and swallowing deficits in PD, which are currently underserved, as these deficits are not fully amenable to standard pharmacological treatments [11-13].

Dopamine modulates the synaptic activity of GABA and glutamate neurons via metabotropic mechanisms [14]. When dopamine is released from presynaptic terminals, it binds with various affinities to five closely related G protein-coupled receptor subtypes, D₁ - D₅. Dopamine receptor subtypes are divided into two overarching families based on structural, pharmacological and biochemical properties. The D₁-like superfamily of dopamine receptors is comprised of D₁ and D₅ receptors and D₂, D₃, and D₄ receptors are members of the D₂-like superfamily. Although all five dopamine receptor subtypes are expressed in the striatum and substantia nigra, the D₁ and D₂ receptor subtypes are most prevalent [14]. However, while it is widely accepted that D₁ and D₂ receptor subtypes are localized to nigrostriatal regions, their contribution to the pathophysiology of PD and related cranial sensorimotor deficits remains unclear and understudied.

Germane to this line of work are animal models, specifically the rat, in which the relationship between aspects of cranial sensorimotor function (e.g., voice and swallowing) and dopaminergic signaling has been examined previously. For example, one study exploring the effects of haloperidol, a D₂ antagonist, on lingual function (measured during a complex tongue protrusive task) and whole body impairment (i.e. cataleptic descent), found differential cranial versus whole body deficits. While haloperidol resulted in both lingual force and whole body impairments that varied as a function of dose (forces decreased; catalepsy increased), there were moderate to weak relationships between force and timing measures and catalepsy, indicating that cranial and whole body/limb sensorimotor function have unique sensitivities to alterations in dopaminergic synaptic transmission [15]. Although methodologies vary, this theme of differential effects of dopaminergic modulation on whole body/limb versus cranial sensorimotor function has been observed in our own lab as well as in other studies [16-19].

Another study investigating laryngeal control examined resting laryngeal muscle activity and the laryngeal adductor response in anesthetized rats during selective antagonism of the D₁ and D₂ receptor subtypes with dopamine antagonists [20]. SCH-23390 antagonizes both D₁ and D₅ receptors, with a higher affinity for the D₁ subtype, while eticlopride antagonizes D₂ and D₃ receptor subtypes, with a higher affinity for the D₂ receptor subtype. D₁ antagonism with administration of SCH-23390 resulted in an increase in resting thyroarytenoid muscle activity as well as shorter laryngeal reflex response latencies, increased amplitudes and reduced excitation. In contrast, D₂ antagonism alone did not alter these physiological and reflexive functions, and combined D₁ and D₂ receptor antagonism

resulted in a similar laryngeal reflex response as was elicited with D₁ antagonism alone. Importantly, similar alterations in laryngeal reflex thresholds and detection have been observed in humans with PD [21]. Overall, these results indicate that D₁ receptors may have a more prominent role in reflexive laryngeal physiology, perhaps by altering the threshold for activation which could have important implications for fine sensorimotor tasks such as voice and speech.

The ultrasonic vocalizations (USVs) of awake behaving rats are also vulnerable to selective antagonism of dopamine receptor subtypes. Rats that received a systemic injection of haloperidol (D₂ receptor antagonist) had decreased bandwidth, complexity, and intensity of USVs compared to controls [22]. Unilateral infusions of the neurotoxin 6-OHDA into the medial forebrain bundle non-selectively disrupts all dopaminergic signaling and results in similar USV deficits (reduced bandwidth, intensity and complexity) as D₂ antagonism alone [23], however, because 6-OHDA is non-selective, the relative contributions of dopaminergic subtypes is unclear. In addition, it has been demonstrated that D₁ and D₂ receptor antagonism results in dose dependent decreases in call rate and complexity (fewer trill-like calls) in amphetamine-induced calling, though the authors did not examine acoustic parameters such as bandwidth, intensity, duration or peak frequency [18]. Taken together, these studies suggest that dopamine receptor subtypes may be implicated in both reflexive and voluntary laryngeal motor control; however, D₁ and D₂ receptor subtypes may have differing degrees of influence on the laryngeal mechanism. Thorough examination of the acoustic components of vocalizations has not been examined, and the differential contributions of D₁ and D₂ receptor modulation on reflexive and voluntary vocalizations requires further exploration.

The purpose of the present study was to examine the effects of dopaminergic (selective and combined D₁ and D₂) antagonism on 50 kilohertz (kHz) USVs. Rats produce ultrasonic vocalizations in the 22 and 50-kHz ranges. These calls have been extensively studied, have been shown to carry semiotic value, have symbolic reference, and are capable of changing the behavior of the signal recipient [24-26]. In addition, there is a wealth of evidence indicating that 50 kHz USV are indicative a positive affect associated with anticipation of reward and/or positive social-emotional contexts, while 22 kHz calls are associated with a negative affect associated with withdrawal or avoidance [16, 24, 27-31]. Thus, human speech/vocalizations and rat ultrasonic vocalizations are both semiotic/semantic in nature, convey emotional states, and are produced via similar mechanisms, modification of egressive airflow with fine sensorimotor control of laryngeal configuration [32, 33]. Given all of this, rat USVs are a valuable model to examine the differential effects of disruptions to dopaminergic signaling that result in cranial (phonatory and communication) and peripheral sensorimotor (whole body) deficits [15, 22, 23, 34, 35] which would otherwise be impossible to study in humans.

We hypothesized that there would be vocalization deficits with both D₁ and D₂ receptor antagonism, with greater deficits resulting from D₁ receptor antagonism, as the D₁ receptor subtype appears to modulate reflexive laryngeal physiology to a greater degree than D₂ receptors [20]. We further expected that degradations of the acoustic signal would be most severe when D₁ and D₂ receptor antagonists are administered in combination, as more dopaminergic receptors are affected. We did not expect a differential response between dopamine receptor subtypes with regard to measures of catalepsy [36]. To this end, rat USVs and whole body catalepsy were analyzed following the administration of selective D₁ and D₂ receptor antagonists, both in isolation and in combination, as well as a vehicle control.

2. Methods

2.1 Animals

Twenty male 4 month-old Long-Evans rats and six female Long-Evans rats (ages 12-15 months) were used in this study (Charles River, Raleigh, NC). Animals were housed in pairs in standard polycarbonate cages with corncob bedding. Lights were maintained on a reverse 12:12 hour light: dark cycle and food and water were available *ad libitum*. All behavioral procedures were conducted during the dark period of the cycle, using partial red illumination. Female Long-Evans rats were used to sexually experience the males and elicit vocalizations, but were not included in any experimental conditions. All experimental procedures were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

2.2 Overview of Testing

Rats were handled daily by the experimenter, habituated to the recording room and sexually experienced for two weeks prior to data collection. Testing consisted of USV recording and cataleptic latency assessment approximately 20 minutes after an intraperitoneal (i.p.) injection of a dopamine antagonist(s) or a vehicle. Rats received no more than 2 injections per week, separated by at least 3 days, thus allowing for a period of drug washout between conditions. Consistent with a within-subjects design, all 20 rats received each of the 4 conditions (SCH-23390, eticlopride, combo, vehicle) only once. The order was of drug administration was randomized for each rat, and the experimenter was masked to the treatment conditions throughout behavioral testing and data analysis.

2.3 Randomized Injections

- (1) *SCH-23390 (D₁)*: This dopamine antagonist (D₁, D₅), which is highly selective for D₁ receptors, was dissolved at 0.4-0.5 mg/ml (free base weight) in saline and injected at 1 ml/kg intraperitoneal (i.p.) for a dose range of 0.4-0.5 mg/kg.
- (2) *Eticlopride (D₂)*: This dopamine antagonist (D₂, D₃), which is highly selective for D₂ receptors, was dissolved at 0.7-0.8 mg/ml (free base weight) in saline and injected i.p. at 1 ml/kg for a dose range of 0.7-0.8 mg/kg.
- (3) *SCH-23390 + Eticlopride (D₁+D₂)*: This injection consisted of both SCH-23390 and eticlopride, targeting both D₁ and D₂ receptor subtypes. Each drug was given at the same dose but as one single i.p. injection with the same total injection volume (i.e. 1 ml/kg).
- (4) *Vehicle*: Rats received a saline injection, which served as a control condition. Saline was injected i.p. at 1 ml/kg.

2.4 Ultrasonic Vocalization Recordings

USVs were recorded with an ultrasonic microphone with a flat frequency response up to 150 kHz and a working frequency response range of 10-180 kHz (CM16, Avisoft, Germany). Vocalizations were recorded at a 214,174 Hz sampling rate, 16 bit depth. The microphone was mounted approximately 15 cm above the rat's home cage. To elicit vocalizations, each male rat was isolated from his cage mate and an estrous female was placed in his home cage. When the male showed signs of interest (sniffing, chasing, mounting), the female was removed, and recording commenced immediately in order to capture latency to first call. Vocalizations were then recorded for 60 seconds after the male began calling.

Two experienced raters, masked to condition, performed offline acoustic analysis 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, frame size of 100%, flat top window selected, and temporal resolution of 75% overlap. A high pass filter was set at 25 kHz to filter extraneous noise. Calls were slowed down by a factor of 11 in order to listen and categorize the call type and down-sampled for analysis. Classification of calls into categories was done by visual and acoustic inspection of each call as has been described previously [22, 23, 34].

To address general levels of arousal or motivation to call, we analyzed call rate (calls per second) and latency to the first call after the female was removed (seconds). We also established overall “call profile” [37] by examining the ratio of simple to complex calls. It has been well established that 50 kHz calls, particularly trill-like frequency modulated calls, are indicative of positive affect associated with rewarding contexts and behaviors [16, 24, 27-31, 38]. Thus, for the purposes of this study, calls were categorized as simple (flat call consisting of a single frequency or very minimal, slow frequency change) or complex (rapid frequency modulation or harmonic component) (Figure 1). Descriptions of the variable call types produced by rats have also been reported in detail elsewhere [22, 37]. The ratio of simple to complex calls was reported as percent complex calls. No 22 kHz calls were observed in any of the experiments.

Detailed acoustic analysis was performed on all call types, collapsed (e.g., simple + complex calls). The following acoustic variables were analyzed using a customized automated program (SASLabPro, Avisoft Bioacoustics, Germany): duration (offset of the signal minus the onset) in seconds, intensity in decibels (dB), bandwidth (maximum minus minimum frequency) in Hertz (Hz), and peak frequency (the frequency at the loudest part of the call) in Hz. Maximum and average values were calculated for each acoustic parameter. Average values reflect the overall performance during a session, while maximum values reflect the “best” performance. This approach was chosen because Parkinsonism often affects average performance to a greater degree than maximal performance on motor tasks [39] and examining only average values may mask differences, as extreme values related to performance are washed out.

2.5 Cataleptic Descent

The cataleptic descent test is a behavioral measure that is sensitive to the effects of dopaminergic antagonism on the initiation of forelimb movement and can be used to index general limb motor impairment [40]. To assess catalepsy, rats were isolated from their cage mate and placed in a standard polycarbonate cage affixed with a stable bar (1 cm in diameter) elevated 8 cm and running parallel to the testing surface. Cataleptic descent time was defined as the duration in seconds between initial forelimb contact with the bar and the return of both forelimbs to the testing surface. Severely cataleptic rats were physically removed from the bar if duration exceeded 2 min (timed out). Latencies during catalepsy were collected for three trials, however only the ‘best performance’, or fastest descent time, following a randomized injection was statistically analyzed, as rats habituated to this test.

2.6 Statistical Analysis

A repeated measures ANOVA (SAS Institute Inc., Cary, NC) was performed on the 4 independent variables (vehicle, D₁, D₂, D₁+ D₂) for each of the dependent variables detailed above. Post hoc comparisons were performed using Fisher's Least Significant Difference. Data that were not normally distributed were log-transformed to better meet the assumptions of ANOVA. The critical value for detecting statistical significance was set at 0.05. Inter and intra-rater reliability was calculated for 10 % of the randomly sampled acoustic data using the intra-class correlation coefficient (ICC). A correlation coefficient of 0.9 or greater was considered reliable.

3. Results

3.1 Rater Reliability

Intra-rater reliability ICC index for bandwidth, duration and intensity were 0.96, 0.99 and 0.99, respectively. Inter-rater reliability ICC index for bandwidth, duration and intensity were 0.98, 0.99 and 1.0, respectively.

3.2 Ultrasonic Vocalization Analysis

3.2.1 Non-acoustic parameters of ultrasonic vocalizations: Call rate, latency to call, & percent complex

—Overall, there was an significant effect of condition on call rate [$F(3,32) = 20.26, p < 0.001$]. Call rate was significantly reduced with D₁ receptor antagonism alone [$t(32) = -5.87, p < 0.001$], D₂ receptor antagonism alone [$t(32) = -4.68, p < 0.001$] and combined D₁ and D₂ receptor antagonism [$t(32) = -6.75, p < 0.001$] compared to the saline condition. Call rate was also significantly reduced in the combined condition compared to D₂ receptor antagonism alone [$t(32) = -2.59, p = 0.014$] (Figure 2A). However, call rate was not significantly different between the D₁ antagonism alone and D₂ antagonism alone conditions [$t(32) = 1.24, p = 0.224$] or in the combined condition compared to D₁ receptor antagonism alone [$t(32) = -1.44, p = 0.16$].

There was an effect of condition on latency to call [$F(3,32) = 18.79, p < 0.001$]. Rats took longer to call following D₁ antagonism [$t(32) = 5.80, p < 0.001$], D₂ antagonism [$t(32) = 4.43, p = 0.001$], and combined D₁ + D₂ antagonism [$t(32) = 6.40, p < 0.001$] compared to the saline condition. Rats also took longer to call in the combination condition compared to the D₂ condition [$t(32) = 2.47, p = 0.02$]. (Figure 2B). There were no significant differences in the latency to call between the D₁ condition and the D₂ [$t(32) = -1.40, p = 0.17$] or combination [$t(32) = 1.18, p = 0.25$] conditions.

The percent of complex calls was significantly affected by condition [$F(3,32) = 4.01, p = 0.016$]. With combined D₁ + D₂ antagonism, rats produced fewer complex calls compared to the saline condition [$t(32) = -3.42, p = 0.002$], the D₁ condition [$t(32) = -2.38, p = 0.023$], and the D₂ condition [$t(32) = -2.59, p = 0.014$] (Figure 2C). There were no significant differences between the percent of complex calls between the saline condition and the D₁ condition [$t(32) = -1.00, p = 0.33$] or D₂ condition [$t(32) = -0.81, p = 0.42$] or between the D₁ and D₂ conditions [$t(32) = -0.19, p = 0.85$].

3.2.2 Acoustic parameters of ultrasonic vocalization: Duration, intensity, bandwidth, and peak frequency

—There was an overall effect of condition on average [$F(3,32) = 10.37, p < 0.001$] and maximum [$F(3,32) = 9.85, p < 0.001$] duration. Rats had significantly shorter average duration of calls with D₁ antagonism [$t(32) = -3.25, p = 0.003$], D₂ antagonism [$t(32) = -2.96, p = 0.006$], and combined D₁ + D₂ antagonism [$t(32) = -5.35, p < 0.001$] compared to the saline. Average duration was also reduced in the combination condition compared to D₁ [$t(32) = -2.29, p = 0.03$] and D₂ [$t(32) = -2.67, p = 0.012$] antagonism (Figure 3A). Similarly, maximum duration was also reduced with D₁ antagonism [$t(32) = -3.28, p = 0.003$], D₂ antagonism [$t(32) = -2.46, p = 0.02$], and combined D₁ + D₂ antagonism [$t(32) = -5.21, p < 0.001$] compared to the saline. Maximum duration was also significantly reduced in the combined D₁ + D₂ antagonism condition compared to D₁ [$t(32) = -2.13, p = 0.041$] and D₂ antagonism alone [$t(32) = -2.95, p = 0.006$] (Figure 3B). There were no significant differences for average [$t(32) = 0.34, p = 0.73$] or maximum [$t(32) = 0.84, p = 0.408$] duration between the D₁ and D₂ conditions.

There was a main effect of condition on maximum [$F(3,32) = 14.30, p < 0.001$] but not average [$F(3,32) = 2.61, p = 0.069$] intensity of vocalizations (Figures 4A, B). Maximum

intensity was reduced in the D₁ [$t(32) = -3.62, p = 0.001$], D₂ [$t(32) = -3.91, p = 0.004$], and combined D₁ + D₂ conditions [$t(32) = -6.25, p < 0.001$] compared to saline, with the D₁ + D₂ combination condition resulting in significantly quieter calls compared to the D₁ [$t(32) = -2.82, p = 0.008$] and D₂ conditions [$t(32) = -2.75, p = 0.009$] (Figure 4B). There was not a significant difference in maximum intensity between the D₁ and D₂ conditions [$t(32) = -0.19, p = 0.85$].

There was an overall effect of condition on average [$F(3,32) = 19.01, p < 0.001$] and maximum [$F(3,32) = 23.35, p < 0.001$] bandwidth. Rats had significantly reduced average bandwidth of calls with D₁ antagonism [$t(32) = -4.74, p < 0.001$], D₂ antagonism [$t(32) = -4.08, p < 0.001$], and combined D₁ + D₂ antagonism [$t(32) = -7.11, p < 0.001$] compared to the saline condition (Figure 5A). Moreover, with D₁ + D₂ antagonism, average bandwidth was also significantly reduced compared to D₁ antagonism [$t(32) = -2.69, p = 0.011$] and D₂ antagonism [$t(32) = -3.44, p = 0.002$]. Similarly, maximum bandwidth was also reduced with D₁ antagonism [$t(32) = -5.84, p < 0.001$], D₂ antagonism [$t(32) = -4.97, p < 0.001$], and combined D₁ + D₂ antagonism [$t(32) = -7.57, p < 0.001$] compared to the saline (Figure 5B) and the combination D₁ + D₂ antagonism resulted in significantly reduced maximum bandwidth compared to D₁ [$t(32) = -2.23, p = 0.033$] and D₂ [$t(32) = -3.12, p = 0.004$] antagonism in isolation. There was no significant difference in average [$t(32) = 0.72, p = 0.474$] or maximum [$t(32) = 0.92, p = 0.362$] bandwidth between the D₁ and D₂ conditions.

There was a significant effect of condition on average [$F(3,32) = 3.93, p = 0.017$] and maximum peak frequency [$F(3,32) = 19.18, p < 0.001$]. Average peak frequency was reduced with D₁ [$t(32) = -3.31, p = 0.002$] and D₂ [$t(32) = -2.12, p = 0.042$], but not combined D₁ + D₂ [$t(32) = -1.79, p = 0.083$] antagonism compared to the saline (Figure 6A). In addition, maximum peak frequency was reduced in the D₁ [$t(32) = -6.44, p < 0.001$], D₂ [$t(32) = -4.10, p < 0.001$], and combination conditions [$t(32) = -6.01, p < 0.001$] compared to saline (Figure 6B). Maximum peak frequency was also reduced with combined D₁ + D₂ antagonism [$t(32) = -2.35, p = 0.025$] and D₁ antagonism [$t(32) = 2.31, p = 0.028$] compared to D₂ antagonism alone. There was no significant difference in average peak frequency between the combination and D₁ [$t(32) = 1.05, p = 0.303$] or D₂ conditions [$t(32) = 0.04, p = 0.971$] or between the D₁ and D₂ conditions [$t(32) = 1.19, p = 0.244$]. Maximum peak frequency was not significantly different between the combination and D₁ antagonism conditions [$t(32) = -0.28, p = 0.782$].

3.3 Catalepsy Descent Time

There was a main effect of condition on catalepsy descent time [$F(3,54) = 23.68, p < 0.001$]. Rats took longer to descend from the catalepsy bar following D₁ receptor antagonism [$t(54) = 5.89, p < 0.001$], D₂ receptor antagonism [$t(54) = 5.60, p < 0.001$], and combined receptor antagonism [$t(54) = 8.07, p < 0.001$] compared to the saline condition. Rats took longer to descend from the catalepsy bar following combined receptor antagonism compared to D₁ receptor antagonism [$t(54) = 2.18, p = 0.034$] and D₂ receptor antagonism [$t(54) = 2.47, p = 0.017$] (Figure 7). There was no significant difference between the D₁ and D₂ conditions [$t(54) = -0.29, p = 0.773$].

4. Discussion

The purpose of this study was to explore the effects of selective dopaminergic receptor antagonism on rat USVs and whole body movement (i.e. catalepsy). USVs were recorded from male rats under 4 different conditions: saline, D₁, D₂, and combined D₁ and D₂ receptor antagonism. Acoustic and non-acoustic parameters (call rate, latency to call, percent complex calls) of USVs as well as whole body catalepsy were analyzed to determine if there were differences between dopamine receptor subtype antagonism on these

parameters. The results support the hypothesis that degradations of acoustic and non-acoustic parameters of vocalizations are most severe following combined receptor antagonism ($D_1 + D_2$) compared with D_1 or D_2 receptor antagonism alone, or the vehicle (saline) condition. As expected, catalepsy was also increased with D_1 , D_2 and combined antagonism compared to saline. Further, the results of this study are consistent with the hypothesis that D_1 antagonism results in more severe acoustic and non-acoustic vocalization deficits, though interpretation of these findings is limited to the dose of each dopamine antagonist that was examined. Importantly, these results indicate that cranial (USV) versus whole body/limb (catalepsy) sensorimotor function are differentially affected by dopamine receptor antagonism.

Detailed acoustic and non-acoustic analysis revealed some clear effects, with acoustic and non-acoustic parameters being differentially vulnerable to D_1 , D_2 , or combined $D_1 + D_2$ antagonism. While D_1 and D_2 antagonism both resulted in definitive changes to USVs (reduced call rate, increased latency to call, decreased duration, intensity, bandwidth and peak frequency), degradations were most pronounced with combined $D_1 + D_2$ antagonism for most parameters. Average and maximum duration, maximum intensity, average and maximum bandwidth were all significantly decreased in the combined condition compared to D_1 or D_2 antagonism alone or saline. Interestingly, maximum peak frequency was more drastically reduced in the D_1 and combined $D_1 + D_2$ antagonism conditions compared to both saline and D_2 antagonism alone, suggesting stronger contribution of this receptor subtype to modulation of this acoustic parameter. Moreover, although latency to call and call rate were both vulnerable to D_1 or D_2 antagonism in isolation and combination, combined antagonism resulted in alterations that were more similar to D_1 antagonism alone (significantly different than D_2 alone, but not D_1), suggesting, again, that the D_1 receptor subtype may contribute to modulating these properties of the acoustic signal to a greater degree. These results indicate that alterations with combined receptor antagonism may reflect the differential effects of D_1 versus D_2 receptor antagonism, with D_1 contributing to a greater degree for those parameters. Examining the effects of each of these receptor subtypes as a function of dose would help to further characterize the nature of each receptor's contribution to components of vocalizations, however, that is beyond the scope of the present investigation.

Importantly, these data indicate that both D_1 and D_2 receptor subtypes contribute to 'typical' ultrasonic vocalization production. Acoustic and non-acoustic parameters were all affected to some degree. This finding is consistent with other work demonstrating that D_1 and D_2 receptor antagonism results in dose dependent decreases in call rate and complexity (fewer trill-like calls) in amphetamine induced calling [18]. Here, we demonstrate that in addition to non-acoustic features such as call rate and complexity, acoustic features such as bandwidth, intensity, duration, and peak frequency are also vulnerable to dopamine receptor subtype antagonism, complementing and extending the previous literature related to the role of dopamine in modulation of vocalizations [18, 22, 23]. Interestingly, we did not observe significant reductions in trill-like complex calls with D_1 or D_2 receptor antagonism alone, only in combination, which is in contrast to previous work [18]. However, methodological differences may account for this discrepancy. For example, in the present study, we used a mating paradigm to induce calling, not amphetamine, which alone results in significant increases in 50 kHz calling [41], and we did not pre-screen out low frequency callers as was done in Wright, et al, 2012 [18]. Correspondingly, decreases in trill-like calls with selective D_1 and D_2 antagonism may have been masked.

There is an abundance of literature suggesting that 50 kHz USVs as well as the dopaminergic mechanisms subserving them are indicative of positive affect and related to rewarding/motivational aspects of behavior [16, 24, 27-31, 38]. Given this, the reductions in

call rate, increased latency to call, and fewer frequency modulated calls may likely reflect alterations of hedonic or appetitive pathways induced by dopaminergic antagonism. However, as previous work has demonstrated that unilateral 6-OHDA lesions result in reductions in complexity without altered call rates as well as altered acoustic features (namely, reductions in bandwidth and intensity), along with disruptions of limb function [23], we believe that the signaling disruptions employed reflect disruptions of cranial sensorimotor function in addition to alterations in reward/motivational aspects (indexed by reduced call rate and complexity). Moreover, as the present study indicates that features such as peak frequency may be differentially vulnerable to dopaminergic signaling, which is reminiscent of previous work demonstrating that D₁ (but not D₂) antagonism alone altered physiological and reflexive laryngeal function, as did combined D₁ and D₂ receptor antagonism [20]. Taken together, these results indicate that D₁ receptors may have a more prominent role in reflexive and voluntary laryngeal physiology. Future studies that are aimed at teasing apart fine sensorimotor laryngeal control from motivation will help clarify the exact underpinnings of USV alterations with disruption of dopamine signaling.

As expected, whole body movement, as measured by catalepsy, was affected with dopamine antagonism. Selective D₁ or D₂ antagonism resulted in significant catalepsy compared to saline, while D₁ or D₂ antagonism in combination led to the longest duration of catalepsy. These data are consistent with the literature demonstrating that antagonism of D₁ or D₂ receptors results in catalepsy [36]. Although isolated D₁ and D₂ receptor antagonism both yielded longer catalepsy times, there were no significant differences between these two conditions. Again, although it is outside the scope of this study, examining catalepsy as a function of dose for comparisons with acoustic USV deficits may have revealed a dose response effect similar to those observed previously that vary indirectly with cranial sensorimotor function [15]. However, the important theme that emerges from these data is that cranial versus whole body/limb sensorimotor function, as measured by acoustic and non-acoustic features of USVs in rats appear to be differentially affected by dopamine receptor antagonism. This feature resonates with previous literature [15-19] and work in our own lab suggesting that while cranial sensorimotor function is vulnerable to alterations in dopaminergic signaling, deficits do not necessarily vary as a function striatal dopamine loss or limb impairment. Discerning the relative contributions of each receptor subtype to cranial sensorimotor function may be important for refining treatment of refractory deficits in PD, such as voice and swallow dysfunction.

In addition to the notable cataleptic behavior with systemic injections of dopamine receptor antagonists, rats did not smell, chase, or mount female rats to the same degree as the vehicle condition, perhaps indicating social and motivational impairments as well as sensorimotor deficits that are mediated by dopamine at the cortical level. Certainly nausea could have contributed to diminished locomotor, mating, and vocalization behavior. While injection dosages were derived from previous research regarding laryngeal control [20], the dosages employed may have been too high in awake animals, as the rats had significantly fewer calls and demonstrated drastic reductions in locomotor behavior when dopamine transmission was altered. However, in light of other studies (e.g. Wright et al, 2012), the alterations in acoustic signaling and catalepsy following dopamine antagonism reported here support the influence of dopamine and its receptor subtypes on both cranial and limb sensorimotor control; the antagonism of D₁ and D₂ receptor subtypes leads to impairments in voluntary motor control throughout the body, albeit to differing degrees depending on the system and possibly related to the motivational/affective influence of the drugs.

While the rat model of ultrasonic vocalization is well established and extensively used for a variety of behavioral studies [42], there are inherent limitations that warrant acknowledgment. Like humans, the larynx has been identified as the sound source for rat

ultrasonic vocalizations [32, 33]. However, human voice is produced through vocal fold vibration and modified by the shape of the vocal tract by articulators, such as the tongue and velopharynx. In the rat, vocalizations are produced by a whistle mechanism that likely requires changes in laryngeal configuration (including adduction) [32, 33]. The modulation of the sound source likely occurs via fine control of the intrinsic laryngeal musculature [33]. Thus, the physiology of vocalization production differs between the rat and the human. However, since we are primarily interested in central nervous system control of vocalizations, these limitations seem reasonable if interpreted responsibly.

We have also noted limitations specific to this study. As mentioned, rats received systemic injections of dopamine antagonists that affected the state of the animal, and possibly altered their motivation for ultrasonic communication. Future studies should consider implementing a dose response curve to determine the optimal level of behavioral and motor impairment. In addition, group average and maximal values are likely influenced by 2 or 3 individual rats who were impressive callers. Pre-screening for high frequency callers in future studies may attenuate the potential impact of “impressive callers” on group averages, by creating a more homogenous group. However, in the present study, we maintain that average and maximal values should be interpreted with slight caution.

5. Conclusion

Degradations in the acoustic signal occurred when D₁ and D₂ receptor subtypes were antagonized, regardless of condition. Rats produced fewer calls, with a delayed initiation of calling behavior and reduced signal complexity. Acoustic degradations were most significant when D₁ and D₂ receptor subtypes were antagonized simultaneously, thus supporting the contributions of both D₁ and D₂ receptor subtypes in vocalization production. In addition, these data suggest that the D₁ receptor subtype may contribute more to certain acoustic parameters than the D₂ receptor subtype (e.g., peak frequency). Perhaps a specialization in receptor roles may be further dissected in future studies with a dose response curve. Given the noticeable increase in catalepsy descent time following D₁, D₂, and combined dopaminergic antagonism, it appears that dopaminergic antagonism impairs not only affective/emotional aspects (latency to call, call rate, call complexity) but also fine complex sensorimotor control tasks, such as vocalization, as well as gross motor control in the limbs. Understanding the role of dopamine receptor subtypes may lead to refinements in the current standard of care for individuals with PD and related cranial sensorimotor disorders, which are currently refractory to traditional pharmacological interventions.

Acknowledgments

The authors would like to thank Drs. Nadine Connor, Gary Weismer, and Glen Levenson for their intellectual and statistical (Levenson) contributions to this project. This project was supported by National Institute on Deafness and Other Communicative Disorders 1P30 DC 010754.

References

1. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003; 24:197–211. [PubMed: 12498954]
2. Bergman H, Deuschl G. Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back. *Mov Disord*. 2002; 17(Suppl 3):S28–40. [PubMed: 11948753]
3. Chesselet MF, Delfs JM. Basal ganglia and movement disorders: an update. *Trends Neurosci*. 1996; 19:417–422. [PubMed: 8888518]
4. Darley FL, Aronson AE, Brown JR. Motor speech signs in neurologic disease. *Med Clin North Am*. 1968; 52:835–844. [PubMed: 5742068]

5. Forrest K, Weismer G, Turner GS. Kinematic, acoustic, and perceptual analyses of connected speech produced by parkinsonian and normal geriatric adults. *J Acoust Soc Am*. 1989; 85:2608–2622. [PubMed: 2745883]
6. Harel B, Cannizzaro M, Snyder PJ. Variability in fundamental frequency during speech in prodromal and incipient Parkinson's disease: a longitudinal case study. *Brain Cogn*. 2004; 56:24–29.10.1016/j.bandc.2004.05.002 [PubMed: 15380872]
7. Ho AK, Iansek R, Marigliani C, Bradshaw JL, Gates S. Speech impairment in a large sample of patients with Parkinson's disease. *Behav Neurol*. 1998; 11:131–137. [PubMed: 11568413]
8. Plowman-Prine EK, Sapienza CM, Okun MS, Pollock SL, Jacobson C, Wu SS, Rosenbek JC. The relationship between quality of life and swallowing in Parkinson's disease. *Mov Disord*. 2009; 24:1352–1358.10.1002/mds.22617 [PubMed: 19425089]
9. Stewart C, Winfield L, Hunt A, Bressman SB, Fahn S, Blitzer A, Brin MF. Speech dysfunction in early Parkinson's disease. *Mov Disord*. 1995; 10:562–565.10.1002/mds.870100506 [PubMed: 8552106]
10. Ferrer I, Martinez A, Blanco R, Dalfo E, Carmona M. Neuropathology of sporadic Parkinson disease before the appearance of parkinsonism: preclinical Parkinson disease. *J Neural Transm*. : 2010.10.1007/s00702-010-0482-8
11. Kompoliti K, Wang QE, Goetz CG, Leurgans S, Raman R. Effects of central dopaminergic stimulation by apomorphine on speech in Parkinson's disease. *Neurology*. 2000; 54:458–462. [PubMed: 10668714]
12. D'Alatri L, Paludetti G, Contarino MF, Galla S, Marchese MR, Bentivoglio AR. Effects of bilateral subthalamic nucleus stimulation and medication on parkinsonian speech impairment. *J Voice*. 2008; 22:365–372.10.1016/j.jvoice.2006.10.010 [PubMed: 17368837]
13. Pinto S, Ozsancak C, Tripoliti E, Thobois S, Limousin-Dowsey P, Auzou P. Treatments for dysarthria in Parkinson's disease. *Lancet Neurol*. 2004; 3:547–556.10.1016/S1474-4422(04)00854-3 [PubMed: 15324723]
14. Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev*. 2011; 63:182–217. 10.1124/pr.110.002642. 10.1124/pr.110.002642 [PubMed: 21303898]
15. Ciucci MR, Connor NP. Dopaminergic influence on rat tongue function and limb movement initiation. *Exp Brain Res*. 2009; 194:587–596.10.1007/s00221-009-1736-2 [PubMed: 19247644]
16. Burgdorf J, Knutson B, Panksepp J, Ikemoto S. Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behav Neurosci*. 2001; 115:940–944. [PubMed: 11508733]
17. Natusch C, Schwarting RK. Using bedding in a test environment critically affects 50-kHz ultrasonic vocalizations in laboratory rats. *Pharmacol Biochem Behav*. 2010; 96:251–259. 10.1016/j.pbb.2010.05.013. 10.1016/j.pbb.2010.05.013 [PubMed: 20493210]
18. 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 (Berl)*. 2013; 225:853–868. 10.1007/s00213-012-2871-1. 10.1007/s00213-012-2871-1 [PubMed: 23052567]
19. Plowman EK, Maling N, Rivera BJ, Larson K, Thomas NJ, Fowler SC, Manfredsson FP, Shrivastav R, Kleim JA. Differential sensitivity of cranial and limb motor function to nigrostriatal dopamine depletion. *Behav Brain Res*. 2013; 237:157–163. 10.1016/j.bbr.2012.09.031. 10.1016/j.bbr.2012.09.031 [PubMed: 23018122]
20. Feng X, Henriquez VM, Walters JR, Ludlow CL. Effects of dopamine D1 and D2 receptor antagonists on laryngeal neurophysiology in the rat. *J Neurophysiol*. 2009; 102:1193–1205. 10.1152/jn.00121.2009. 10.1152/jn.00121.2009 [PubMed: 19535485]
21. Hammer MJ, Barlow SM. Laryngeal somatosensory deficits in Parkinson's disease: implications for speech respiratory and phonatory control. *Exp Brain Res*. 2010; 201:401–409.10.1007/s00221-009-2048-2 [PubMed: 20012947]
22. 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. *Behav Brain Res*. 2007; 182:284–289.10.1016/j.bbr.2007.02.020 [PubMed: 17397940]

23. 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. *Behav Neurosci.* 2009; 123:328–336.10.1037/a0014593 [PubMed: 19331456]
24. Brudzynski SM, Pniak A. Social contacts and production of 50-kHz short ultrasonic calls in adult rats. *J Comp Psychol.* 2002; 116:73–82. [PubMed: 11926686]
25. Wöhr M, Houx B, Schwarting RK, Spruijt B. Effects of experience and context on 50-kHz vocalizations in rats. *Physiol Behav.* 2008; 93:766–776. 10.1016/j.physbeh.2007.11.031. 10.1016/j.physbeh.2007.11.031 [PubMed: 18191963]
26. Bialy M, Rydz M, Kaczmarek L. Precontact 50-kHz vocalizations in male rats during acquisition of sexual experience. *Behav Neurosci.* 2000; 114:983–990. [PubMed: 11085613]
27. 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; 182:274–283.10.1016/j.bbr.2007.03.010 [PubMed: 17449117]
28. Burgdorf J, Kroes RA, Moskal JR, Pfaus JG, Brudzynski SM, Panksepp J. Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. *J Comp Psychol.* 2008; 122:357–367. 10.1037/a0012889. 10.1037/a0012889 [PubMed: 19014259]
29. Burgdorf J, Panksepp J, Moskal JR. Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. *Neurosci Biobehav Rev.* 2011; 35:1831–1836. 10.1016/j.neubiorev.2010.11.011. 10.1016/j.neubiorev.2010.11.011 [PubMed: 21144859]
30. Brudzynski SM. Ultrasonic calls of rats as indicator variables of negative or positive states: acetylcholine-dopamine interaction and acoustic coding. *Behav Brain Res.* 2007; 182:261–273.10.1016/j.bbr.2007.03.004 [PubMed: 17467067]
31. Brudzynski SM. Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. *Curr Opin Neurobiol.* 2013 10.1016/j.conb.2013.01.014. 10.1016/j.conb.2013.01.014
32. Riede T. Subglottal pressure, tracheal airflow, and intrinsic laryngeal muscle activity during rat ultrasound vocalization. *J Neurophysiol.* 2011; 106:2580–2592.10.1152/jn.00478.2011 [PubMed: 21832032]
33. Johnson AM, Ciucci MR, Russell JA, Hammer MJ, Connor NP. Ultrasonic output from the excised rat larynx. *J Acoust Soc Am.* 2010; 128:EL75–9.10.1121/1.3462234 [PubMed: 20707418]
34. Ciucci MR, Vinney L, Wahoske EJ, Connor NP. A translational approach to vocalization deficits and neural recovery after behavioral treatment in Parkinson disease. *J Commun Disord.* 2010; 43:319–326.10.1016/j.jcomdis.2010.04.004 [PubMed: 20434728]
35. Russell JA, Ciucci MR, Connor NP, Schallert T. Targeted exercise therapy for voice and swallow in persons with Parkinson's disease. *Brain Res.* 2010; 1341:3–11.10.1016/j.brainres.2010.03.029 [PubMed: 20233583]
36. Morelli M, Di Chiara G. Catalepsy induced by SCH 23390 in rats. *Eur J Pharmacol.* 1985; 117:179–185. [PubMed: 2866970]
37. 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. 10.1007/s00213-010-1859-y. 10.1007/s00213-010-1859-y [PubMed: 20443111]
38. Knutson B, Burgdorf J, Panksepp J. Ultrasonic vocalizations as indices of affective states in rats. *Psychol Bull.* 2002; 128:961–977. [PubMed: 12405139]
39. Abbruzzese G, Berardelli A. Sensorimotor integration in movement disorders. *Mov Disord.* 2003; 18:231–240.10.1002/mds.10327 [PubMed: 12621626]
40. Sanberg PR, Bunsey MD, Giordano M, Norman AB. The catalepsy test: its ups and downs. *Behav Neurosci.* 1988; 102:748–759. [PubMed: 2904271]
41. 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; 197:205–209.10.1016/j.bbr.2008.08.037 [PubMed: 18809437]

42. Cenci MA, Whishaw IQ, Schallert T. Animal models of neurological deficits: how relevant is the rat? *Nat Rev Neurosci.* 2002; 3:574–579.10.1038/nrn877 [PubMed: 12094213]

We examine the effects of dopamine receptor subtypes on ultrasonic vocalization
D1 and D2 dopamine receptor subtypes differentially modulate ultrasonic vocalization
Antagonism of D1 receptors alters acoustic parameters to a greater degree than D2
Limb and cranial sensorimotor systems dissociate with dopamine receptor antagonism

Figure 1.
Representative ultrasonic vocalization call types. A Representative Simple Call B.
Representative Complex Call. Relative intensity is represented by the darkness of the call.

Figure 2.

2A Call rate in calls/second, 2B Latency to call in seconds, 3C Percent of complex calls in the saline, D₁, D₂, and combination (D₁ + D₂) antagonism conditions. Data presented as means and SEM. Bar indicates statistical significance at the $p < 0.05$ level.

Figure 3.

3A Average duration in seconds and 3B maximum duration in seconds in the saline, D₁, D₂, and combination (D₁ + D₂) antagonism conditions. Data presented as means and SEM. Bar indicates statistical significance at the $p < 0.05$ level.

Figure 4.

4A Average intensity in decibels 4B maximum intensity in decibels in the saline, D₁, D₂, and combination (D₁ + D₂) antagonist conditions. Data presented as means and SEM. Bar indicates statistical significance at the $p < 0.05$ level.

Figure 5.

5A Average bandwidth in hertz and 5B maximum bandwidth in hertz in the saline, D₁, D₂, and combination (D₁ + D₂) antagonism conditions. Data presented as means and SEM. Bar indicates statistical significance at the $p < 0.05$ level.

Figure 6. 6A Average peak frequency in hertz and 6B maximum peak frequency in hertz in the saline, D₁, D₂, and combination (D₁ + D₂) antagonism conditions. Data presented as means and SEM. Bar indicates statistical significance at the $p < 0.05$ level.

Figure 7. Average catalepsy time in seconds for the saline, D₁, D₂, and combination (D₁ + D₂) antagonism conditions. Data presented as means and SEM. Bar indicates statistical significance at the $p < 0.05$ level.