HHS Public Access

Author manuscript

Behav Brain Res. Author manuscript; available in PMC 2023 February 10.

Published in final edited form as:

Behav Brain Res. 2022 February 10; 418: 113642. doi:10.1016/j.bbr.2021.113642.

Manipulation of Vocal Communication and Anxiety through Pharmacologic Modulation of Norepinephrine in the *Pink1−/−* Rat Model of Parkinson Disease

Jesse D. Hoffmeister[a,b], **Cynthia A. Kelm-Nelson**[b], **Michelle R. Ciucci**[a,b,c]

[a]Department of Communication Sciences and Disorders, University of Wisconsin-Madison; 1975 Willow Drive, Madison, WI 53706 USA

[b]Department of Surgery, Division of Otolaryngology-Head & Neck Surgery, University of Wisconsin-Madison, 600 Highland Avenue, Madison, Wisconsin 53792-7375 USA

[c]Neuroscience Training Program, University of Wisconsin-Madison, 9531 WIMR II, 1111 Highland Avenue, Madison, WI 53705 USA

Abstract

Vocal deficits and anxiety are common, co-occurring, and interacting signs of Parkinson Disease (PD) that have a devastating impact on quality of life. Both manifest early in the disease process. Unlike hallmark motor signs of PD, neither respond adequately to dopamine replacement therapies, suggesting that their disease-specific mechanisms are at least partially extra-dopaminergic. Because noradrenergic dysfunction is also a defining feature of PD, especially early in the disease progression, drug therapies targeting norepinephrine are being trialed for treatment of motor and non-motor impairments in PD. Research assessing the effects of noradrenergic manipulation on anxiety and vocal impairment in PD, however, is sparse. In this pre-clinical study, we quantified the influence of pharmacologic manipulation of norepinephrine on vocal impairment and anxiety in *Pink1*−/− rats, a translational model of PD that demonstrates both vocal deficits and anxiety. Ultrasonic vocalization acoustics, anxiety behavior, and limb motor activity were tested twice for each rat: after injection of saline and after one of three drugs. We hypothesized that norepinephrine reuptake inhibitors (atomoxetine and reboxetine) and a β receptor antagonist (propranolol) would decrease vocal impairment and anxiety compared to saline, without affecting spontaneous motor activity. Our results demonstrated that atomoxetine and reboxetine decreased anxiety behavior.

Atomoxetine also modulated ultrasonic vocalization acoustics, including an increase in vocal intensity, which is almost always reduced in animal models and patients with PD. Propranolol did

Please address correspondence to Jesse Hoffmeister, Ph.D., hoff0692@umn.edu, Present Address: 420 Delaware Street SE, Minneapolis, MN 55455.

CRediT authorship contribution statement

Jesse Hoffmeister: Conceptualization, Methodology, Investigation, Writing – Original Draft, Visualization **Cynthia Kelm-Nelson**: Methodology, Resources, Writing-Reviewing and Editing. **Michelle Ciucci:** Conceptualization, Resources, Writing- Reviewing, Funding Acquisition

not affect anxiety or vocalization. Drug condition did not influence spontaneous motor activity. These studies demonstrate relationships among vocal impairment, anxiety, and noradrenergic systems in the *Pink1*−/− rat model of PD.

Keywords

Parkinson Disease; Rat; Pink1; Ultrasonic Vocalization; Anxiety; Norepinephrine

1.1: Introduction

Deficits in vocal communication and anxiety are common signs of Parkinson Disease (PD) that share a disease-specific timeline, influence one another, and are poorly understood and chronically undertreated. The locus coeruleus-noradrenergic system strongly influences both anxiety and vocal communication. Because noradrenergic dysfunction is central to parkinsonian neural pathology, simultaneous exploration of noradrenergic dysfunction relative to both vocal deficits and anxiety may lead to more-nuanced understanding of the disease-specific neural mechanisms that drive these impairments.

In addition to experiencing hallmark motor signs of bradykinesia, tremor, and postural instability, vocal communication impairment is present in up to 90% of individuals with PD [1–7], and up to 55% are diagnosed with anxiety [8,9]. These two common, “non-hallmark” signs of PD are distinct from hallmark motor signs in that they occur early in the disease process, often prior to the onset of classical motor deficits such as tremor and bradykinesia [8,10–15]. In further contrast to classical motor deficits, vocal impairment and anxiety have limited responses to standard pharmacologic interventions, such as levodopa, suggesting that their disease-specific mechanisms are at least partially extra-dopaminergic [16–21]. Additionally, behavioral interventions such as Lee-Silverman Voice Treatment (LSVT LOUD®), and cognitive-behavioral therapy for anxiety, result in incomplete and transient improvements [16,22–24]. Unfortunately neurosurgical interventions, such as deep brain stimulation, also have a limited effect, and can even exacerbate vocal impairment and anxiety [25,26]. As a consequence, vocal impairment and anxiety in PD remain undertreated.

Multiple lines of research have begun to converge on the fact that dysfunction of norepinephrine (NE) in the central nervous system is associated with both vocal impairment [27–35] and anxiety [36–38]; additionally, preliminary evidence in rat models of PD from our laboratory has shown that anxiety behaviors and vocal communication are correlated[39]. These observations further distinguish vocal impairment and anxiety from the hallmark motor signs of PD. In response, investigations into pharmacologic manipulation of NE in PD have begun [28,29,35,40,41].

Studies of pharmacologic modulation of NE have resulted in improvements to both motor and non-motor aspects of PD [28,29]. These include modification of attention, hallucination, cognitive impairments, freezing of gait, and response inhibition in humans [33,41]. In addition, it has been shown that modulation of NE in non-parkinsonian rats influences acoustic features of vocalization [42,43]. Two norepinephrine reuptake inhibitors that have

shown particular promise for modulating symptoms of PD are atomoxetine and reboxetine, both of which block NE transporter. Treatment with atomoxetine has been associated with improved executive function [40] and reduced indices of depression [44] in PD, suggesting modulation of multiple neural systems. Further, atomoxetine has been found to significantly reduce levels of anxiety in certain populations [45]. Reboxetine also improves depression, as well as motor performance in human and animal studies [28,35] of PD, and there is emerging evidence that reboxetine is effective in the treatment of panic disorder and anxiety [46,47].

Noradrenergic receptor modulation is another method of altering central NE functions that shows promise for addressing some parkinsonian deficits. The β-adrenoreceptor antagonist, propranolol, is most-commonly prescribed for the treatment of hypertension. Because of its sympatholytic properties, researchers have hypothesized that propranolol might be used to treat anxiety since the mid 20th century [48]. Administration of propranolol has resulted in decreased anxiety behavior in rodents [49–51], as well as decreased anxiety in some types of anxiety disorders in humans [48,52–55]. In addition, the use of propranolol modifies vocal communication not only in wildtype (WT) control rats [42,43] and in healthy humans[56], but has also resulted in improvement in levodopa-induced limb and trunk dyskinesias in humans with PD [57], and levodopa-induced trunk, limb, and orolingual dyskinesias in rat models of the disease [58]. Research on the effects of NE manipulation in PD on anxiety and vocal impairment, however, is sparse, and research on interactions among NE, vocal impairment, and anxiety is absent from the literature.

In this pre-clinical study, we measured vocal communication and anxiety following administration of three different drugs that modulate NE in rats with a knockout of the *Pink1* gene, an established model of PD [59–63]. The *Pink1*−/− rat is based on a genetic form of early and progressive PD (PARK6) that is nearly identical to idiopathic PD [64]; the *Pink1*−/− rat has been well-validated as a model of vocal communication impairment in PD [61,63,65,66], and preliminary research from our laboratory has demonstrated increased anxiety in this model compared to WT controls [39,62]. Importantly, disruptions in monoaminergic systems in the *Pink1*−/− rat have also been identified. Changes to brainstem norepinephrine in the *Pink1*−/− rat have been correlated with vocalization deficits[39,63,65], oromotor deficits[67], and anxiety[39]. We hypothesized that NE reuptake inhibitors (atomoxetine and reboxetine) and a β-adrenoreceptor antagonist (Propranolol) would decrease vocal impairment and anxiety, but would not change spontaneous motor activity. Further, we hypothesized that the relationships between anxiety and vocal impairment would be altered by each drug. Atomoxetine, reboxetine and propranolol were chosen because they have been used to treat anxiety through NE mechanisms. Other anxiolytic drugs (*i.e.* benzodiazepines) were not chosen because they are often associated with motor impairment [68].

While these drugs have been used to investigate and to treat other non-motor signs of PD, their effect on vocal impairment and anxiety remains unstudied. Because these drugs target NE functions with a high degree of specificity and have been shown to have an influence on other non-motor signs of PD, the study of their effect on vocal impairment and anxiety would clarify the role of NE functions in vocal impairment and anxiety in PD.

These drugs in particular were also chosen because they are FDA-approved, facilitating clinical translation, and because they are not associated with fatigue or reductions in motor coordination.

2: Methods

2.1: Study Design

Three separate, within-subjects experiments were conducted to assess the influence of propranolol, atomoxetine, or reboxetine on vocal communication and anxiety behavior. Each rat was injected once with saline, and once with one of the three drugs, with each rat thus serving as its own control.

2.2: Animals

Thirty-six eight-month-old *Pink1*−/− Long-Evans rats were randomly assigned to one of three groups: atomoxetine, reboxetine, and propranolol (n=12 rats per group). Sample size was determined from power calculations based on vehicle-dose differences for atomoxetine reported by Robinson(2008) [69]. To account for potential attrition (e.g., not vocalizing) and to pair-house the rats, a sample size of 12 rats was predicted to detect differences at the 0.05 significance level with 90% power. The 8-month age was chosen to reflect the age at which anxiety in the *Pink1*−/− rat is most apparent[39,62]. Each rat underwent anxiety testing on the elevated plus maze followed immediately by recording of ultrasonic vocalizations (USVs) after both saline injection and drug injection. Saline and drug testing conditions were separated by three weeks in order to reduce habituation to the testing apparatus [70–72], and to ensure adequate drug washout [42,69,73]. The order of saline and drug condition was randomly assigned using a random number generator and counterbalanced in each drug group to account for potential order effects. Behavioral testing following both saline and drug administration occurred at the same interval following injection, and was determined by the half-life of the drug (see below). In addition, 12 female Long-Evans rats were used to elicit USVs (protocol below). These female rats were continually housed in the colony maintained by our laboratory for the purpose of ultrasonic vocalization elicitation in several ongoing studies. Each experimental rat was exposed to each female rat in the colony during the acclimation period. All animals were obtained from SAGE Labs (Envigo, Boyertown, PA). Rats were housed in pairs in the Biomedical Research Model Services facilities of the UW School of Medicine and Public Health on a 12-hour reversed light-cycle. All behavioral testing was under red light during the dark period when rats are most active. Rats were handled and weighed weekly until testing and throughout the duration of the study. Standard animal husbandry and other handling practices and procedures were implemented, related to animal health monitoring, diet, cage, environmental control, and general exercise in accordance with institutional guidelines regarding animal experimentation. All procedures were approved by the University of Wisconsin-Madison School of Medicine and Public Health Animal Care and Use Committee (IACUC; protocol M005177-R01-A04) and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, Eight Edition [74].

2.3: Experimental Procedure

Pink1−/− rats underwent testing of anxiety followed immediately by recording of mating paradigm-elicited USVs for acoustic analysis. The immediate recording of ultrasonic vocalizations was performed in order to ensure that USVs could be considered relative to anxiety state, which may vary over time. After USV recording, rats also underwent assessment of spontaneous motor activity with the cylinder test. Each rat was tested at two time points at 8-months of age: once following saline injection and once following injection of atomoxetine, reboxetine, or propranolol. Order of saline versus drug was randomized and counterbalanced for each drug cohort. Time points were separated by three weeks in order to reduce the confound of repeated exposure to the elevated plus maze in a short time period[70].

2.4: Drugs and Drug Administration

Atomoxetine (Sigma-Aldrich, St. Louis, MO, PubChem SID 329831496) was dissolved in sterile isotonic saline at 3mg/ml, for a dose of 1.5 mg/kg, and injected intraperitoneally. Behavioral testing was performed 30 minutes after the injection. Reboxetine (MedChem Express, Monmouth Junction, NJ, PubChem SID 210280742) was dissolved in sterile isotonic saline at 30mg/ml for a dose 30 mg/kg and injected intraperitoneally, with behavioral testing performed 30 minutes after the injection. Propranolol (Sigma-Aldrich, St. Louis, MO, PubChem CID: 62882) was also dissolved in sterile isotonic saline at 3mg/ml, for a dose 3 mg/kg and injected intraperitoneally. Behavioral testing was performed 20 minutes after the injection. Saline vehicle was injected intraperitoneally at the same volumes as paired drugs for each rat based on weight on the day of testing. Behavioral testing was completed at the same interval following injection of saline as for paired drugs. Order of drug and saline were randomized and counterbalanced such that half of the rats received saline first, and half received the drug first. Drug doses were chosen based on clinically translatable doses in humans adjusted for species differences for atomoxetine and propranolol [40,41,75–77], and on doses that have shown changes to vocalization and other behaviors in WT rats for atomoxetine, propranolol and reboxetine [35,42,73,78,79]. Doses of reboxetine were higher than human equivalent doses because lower doses result in limited changes to rat behavior [35,46,47,78,79].

2.5: Behavioral Assays

2.5.1: Elevated Plus Maze: At the designated time following injection, rats were placed on the elevated plus maze for the assessment of anxiety behavior [70,80–82]. The elevated plus maze was chosen instead of other methods of anxiety assessment because of its frequent use in the measurement of anxiety, facilitating comparison with the extant literature[83]. The plus-shaped platform was constructed with 4 equally sized arms. Two arms are open with no walls (50×10cm), and the remaining two arms (opposite one another) have walls on 3 of 4 sides and an open top(50×10×50cm). Rats can enter each arm from a square in the center of the platform. Each arm is accessible from a square area in the center of the platform (Figure 1). The rats were placed in the center of the maze under red light facing an open arm, and were video-recorded for 5 minutes. Movement was tracked and analyzed using EthoVision software(Noldus Ethovision XT (Wageningen, Netherlands)).

Outcome variables were total entries into open and closed arms and total time spent in open and closed arms in seconds. Increased time and frequency of entry into closed arms represent increased anxiety. Because repeated exposure to the plus maze in a short time interval can result in reduced exploration of open arms, thus inflating estimates of anxiety behavior, testing time points were separated by 3 weeks[70–72].

2.5.2: Ultrasonic Vocalization Recording—Ultrasonic vocalizations (USVs) are produced in the 50-kiloHertz (kHz) range in a variety of conditions, especially to initiate and maintain conspecific contact. These USVs are complex and are produced in patterns. Modulation of some acoustic features of USVs, including mean peak frequency, bandwidth, and complexity, results in increased approach behavior of conspecifics [84], and can be considered to be at least partially goal directed[85–90]. In rat models of PD and other neurologic diseases, acoustic analysis of USVs is commonly used to assay vocal deficits[42,61,63,84,91–93]. To measure vocal communication in the current study, the following paradigm was used to elicit and record USVs: immediately following anxiety assessment on the elevated plus maze, test rats were placed in their home cage under a microphone attached to an ultrasonic recording system (CM16, Avisoft, Germany) with a 10–180kHz working frequency response range set to a 16-bit depth and 250kHz sampling rate. A female conspecific in estrus was then be placed in the male rat's home cage. After the male rat showed interest in the female, the female was removed. USVs from the male rat were recorded for 90 seconds and were analyzed offline using DeepSqueak 2.6 [94] in MATLAB (version 9.5.0.944444[R2018b]; The MathWorks Inc., Natick, MA). DeepSqueak outputs a spectrogram and identifies calls using a pre-trained neural network [94–97]. Following automatic detection, calls were visually inspected and noise events misclassified as calls were rejected [95,96]. Calls were categorized as either simple or complex based on visual inspection of the spectrogram. Simple calls were defined as having a flat contour without repetitive frequency modulation [93]; remaining calls were categorized as complex (Figure 2).

The following parameters were extracted: total number of calls, proportion of complex calls (number of complex calls divided by total calls), intensity as measured by power spectral density in decibels(dB)/kilohertz(kHz), duration in seconds, principal frequency (the median frequency of the call contour) in kHz, sinuosity (a measure reflective of call complexity defined as the ratio between the Euclidean distance of a straight line and curvilinear length along a curve; simple calls have sinuosity near 1, and complex calls have a greater sinuosity), call bandwidth in kHz, and tonality (1 minus the geometric mean of the power spectrum divided by the arithmetic mean) as a measure of distinguishability of the signal of the contour from noise (higher numbers indicate less background noise present in the call). Data from individual calls were averaged for each animal at each recording timepoint for statistical analyses.

2.5.3: Cylinder Test—Spontaneous movement was assessed via the cylinder test [98], as differences in spontaneous movement could potentially influence exploratory behavior on the elevated plus maze. Immediately following USV testing, rats were placed in a 30cm by 20cm cylinder that was clear and positioned vertically to encourage rearing and vertical

exploratory behavior for the assessment of general motor function. The cylinder was placed on top of a clear plastic box and rat behavior was videorecorded for two minutes. Videos were analyzed off-line to assess the number of forelimb movements, hindlimb movements, rears, and lands.

2.6: Statistical Analysis

All analyses were completed separately for each drug experiment; outcomes were not compared across drug experiments. For each drug, paired t-tests were used to compare spontaneous movement, anxiety, and USV acoustic outcome measures between drug group and saline group. USV analysis was performed with all calls, regardless of classification, for primary analysis, as well as with simple calls and complex calls separately in post-hoc secondary analysis. Primary analysis of all calls regardless of call categorization was performed because noradrenergic manipulation was anticipated to influence vocalization globally. Secondary analysis of the broad categories of simple and complex calls was performed to account for inherent differences between these categories, such as bandwidth. Linear mixed effects regression models were used in exploratory analysis to assess the interaction between drug condition and anxiety level, as measured by time spent in closed arms of the maze, on USV outcomes. Corrections for multiple comparisons were not performed due to the exploratory nature of this work and associated type II statistical error. Statistical analyses were performed with a significance level of 0.05 using software R (version 3.6.0) and SAS (version 9.4).

3: Results:

3.1: Anxiety

3.1.1: Propranolol—Anxiety outcomes of time spent in open arms of the plus maze ($t(11)=0.13$, $p=0.9$), time spent in closed arms of the plus maze ($t(11)=0.66$, $p=0.52$), number of entries into open arms of the plus maze ($t(11)=0.57$, $p=0.58$), and number of entries into closed arms of the plus maze ($t(11)=1.6$, $p=0.14$) did not differ between saline and propranolol conditions.

3.1.2: Atomoxetine—There was a significant decrease in the amount of time spent in closed arms of the maze with atomoxetine compared to saline (mean difference=−48 seconds, $t(9)=3.26$, $p=0.01$). There was also a significant decrease in the number of entries into closed arms of the maze with atomoxetine compared to Saline (mean difference =2.9 entries, $t(9)=2.69$, $p=0.025$). These findings indicate decreased anxiety-like behavior with atomoxetine compared to saline. Time spent in open arms of the plus maze ($t(9)=1.11$, $p=0.30$), and entries into open arms of the plus maze ($t(9)=1.17$, $p=0.27$) did not differ between saline and atomoxetine conditions. Sample size for elevated plus maze measurements was 10 rats, as one rat fell from the maze during the first testing timepoint.

3.1.3: Reboxetine—There was a significant decrease in amount of time spent in closed arms of the plus maze with reboxetine compared to saline (mean difference=22.5 seconds, $t(11)=2.33$, $p=0.04$) There was also a significant decrease in the number of closed arm entries with reboxetine compared to saline (mean difference=4 entries, $t(11)=3.63$, $p=0.004$).

There were no significant differences in the number of open arm entries (t(11)=1.28, p=0.23), or time spent in open arms of the maze (t(11)=−1.33, p=0.21) between saline and reboxetine conditions.

3.2: Ultrasonic Vocalization

The primary USV outcomes were measured on all calls for each rat. Exploratory analysis of sub-categories of complex and simple calls was then conducted. Simple calls were defined as calls that did not contain oscillatory pitch modulation or step-wise modulation. Complex calls represented all non-simple calls.

3.2.1: Propranolol—There were no significant changes in the USV parameters of call duration (t(11)=−1.47, p=0.17), principal frequency (t(11)=−0.61, p=0.55), tonality (t(11)=0.28, p=0.78), delta frequency (t(11)=0.20, p=0.84), mean power(t(11)=0.02, p=0.98), or sinuosity (t(11)=0.88, p=0.40) between saline and propranolol conditions.

There were no significant differences in the total number of calls (t(11)=−0.05, p=0.96), or proportion of complex calls (t(11)=−0.35, p=0.73) between saline and propranolol conditions (see Table 1). For simple calls, there were no significant changes in call duration (t(11)=1.28, p=0.23), principal frequency (t(11)=−0.75, p=0.47), tonality (t(11)=0.41, p=0.69), delta frequency (t(11)=0.14, p=0.89), mean power (t(11)=0.89, p=0.39), or sinuosity (t(11)=0.74, p=0.48). For complex calls, there were also no significant changes in call duration (t(11)=−1.43, p=0.18), principal frequency (t(11)=−0.54, p=0.6), tonality (t(11)=0.44, p=0.67), delta frequency (t(11)=0.18, p=0.85), mean power (t(11)=0.3, p=0.77), or sinuosity (t(11)=1.28, p=0.23).

3.2.2: Atomoxetine—There was a significant decrease in the principal frequency with atomoxetine compared to saline (mean difference = 3.06 kHz, t(10)=2.85, p=0.017). Tonality increased significantly with atomoxetine compared to saline (mean difference=0.033, t(10)=2.47, p=0.03). There was a significant decrease in the delta frequency (bandwidth) with atomoxetine compared to saline (mean difference=2.43, t(10)=2.35, p=0.041), as well as a significant increase in mean power (mean difference=1.9, t(10)=2.92, p=0.015). Finally, there was a significant decrease in sinuosity for complex calls with atomoxetine compared to saline (mean difference=0.41, t(10)=2.35, p=0.04). There was no change in duration (t(10)=−0.91, p=0.39) between saline and atomoxetine conditions.

There were no significant differences in the total number of calls (t(10)=−0.45, p=0.67) or the proportion of complex calls (t(10)=0.79, p=0.45) between saline and atomoxetine conditions (Table 1). For complex calls, there was a there was a significant decrease in the principal frequency with atomoxetine compared to saline condition (mean difference = 2.51 kHz, t(10)=2.23, p=0.04975). There were no differences in duration (t(10)=0.95, p=0.37), tonality (t(10)=1.45, p=0.18), delta frequency (t(10)=0.79, p=0.45), mean power (t(10)=2.05, p=0.07), or sinuosity (t(10)=1.47, p=0.17).

For simple calls, there were no significant differences in duration (t(9)=0.39, p=0.71), principal frequency (t(9)=1.94, p=0.08), tonality (t(9)=1.72, p=0.12), delta frequency (t(9)=1.43, p=0.19), mean power (t(9)=1.91, p=0.09) or sinuosity (t(9)=1.02, p=0.09).

3.2.3: Reboxetine—There were no significant differences between saline and reboxetine for USV outcomes of duration (t(11)=0.75, p=0.47), principal frequency (t(11)=1.85, p=0.09), tonality (t(11)=0.51, p=0.62), delta frequency (t(11)=1.79, p=0.10), mean power (t(11)=1.76, p=0.11), or sinuosity (t(11)=0.85, p=0.4).

There was a significant decrease in the total number of calls with reboxetine compared to saline (mean difference=52.17 calls, t(11)=3.15, p=0.01). There was no significant difference in the proportion of complex calls between saline and reboxetine (t(11)=1.37, p=0.18) (Table 1). For complex calls, there was a significant decrease in the mean power with Reboxetine compared to saline (mean difference=1.79dB, t(11)=2.68, p=0.02). There was no difference in duration (t(11)=0.39, p=0.7), principal frequency (t(11)=1.73, p=0.11), tonality (t(11)=1.72, p=0.11), delta frequency (t(11)=1.99, p=0.07), or sinuosity (t(11)=0.68, p=0.51).

For simple calls, there was a significant decrease in the Principal Frequency of Simple calls with Reboxetine compared to saline (mean difference=3.56, t(11)=2.22, p=0.048). There were no differences in duration (t(11)=2.09, p=0.06), tonality (t(11)=0.6, p=0.56), delta frequency (t(11)=−0.87, p=−.4), mean power (t(11)=0.37, p=0.72), or sinuosity (t(11)=1.19, p=0.26).

3.3: Interactions between Drug Condition and Anxiety on Ultrasonic Vocalization outcomes

3.3.1: Propranolol—The relationships between anxiety, as measured by time spent in closed arms of the elevated plus maze, and call duration (β=−0.0001223, t=0.35, p=0.66), anxiety and tonality (β=0.00027, t=−0.582, p=0.567), anxiety and principal frequency (β=0.021, t=0.33, p=0.75), anxiety and sinuosity (β=0.004, t=−0.76, p=0.46), anxiety and mean power (β=0.0009, t=−0.044, p=0.97), and anxiety and delta frequency (β=−0.024, t=−0.65, p=0.56) were not significantly influenced by drug condition.

3.3.2: Atomoxetine—The relationships between anxiety, as measured by time spent in closed arms of the elevated plus maze, and call duration (β=−0.00013, t=−1.56, p=0.13), anxiety and tonality (β=−0.0002, t=0.48, p=0.64), anxiety and principal frequency (β=−0.039, t=−1.1, p=0.3), anxiety and sinuosity (β=−0.003, t=−0.59, p=0.57), anxiety and mean power (β=0.03, t=1.22, p=0.25), and anxiety and delta frequency (β=−0.01, t=−0.44, p=0.67) were not significantly influenced by atomoxetine.

3.3.3: Reboxetine—The relationships between anxiety, as measured by time spent in closed arms of the elevated plus maze, and call duration (β=−0.00006, t=−0.51, p=0.61), anxiety and tonality (β=−0.0005, t=−1.08, p=0.3), anxiety and principal frequency (β=0.011, t=0.27, p=0.79), anxiety and sinuosity (β=0.0029, t=0.61, p=0.55), anxiety and mean power(β=−0.02, t=−0.86, p=0.41), and anxiety and delta frequency (β=−0.001, t=−0.029, p=0.98) were not significantly influenced by reboxetine.

3.4: Cylinder

3.4.1: Propranolol—Cylinder outcomes of number of hindlimb movements (t(10)=0.74, p=0.47), forelimb elevations (t(10)=1.03, p=0.33), forelimb returns to the floor (t(10)=0.73, p=0.48), rears (t(10)=0.41, p=0.69), lands (t(10)=0.18, p=0.86), and rears plus lands (t(10)=0.3, p=0.77) all did not differ by between saline and propranolol. One rat was not video-recorded.

3.4.2: Atomoxetine—Cylinder outcomes of number of hindlimb movements (t(10)=1.55, p=0.15), number of forelimb elevations (t(10)=1.07, p=0.31), number of forelimb returns to the floor (t(10)=0.41, p=0.69), number of rears (t(10)=0.98, p=0.35), number of lands (t(10)=0.74, p=0.48), and number of rears plus lands (t(10)=0.87, p=0.41) did not differ between saline and atomoxetine. One rat expired prior to study initiation.

3.4.3: Reboxetine—Cylinder outcomes for the number of hindlimb movements (t(11)=2.034, p=0.067), number of forelimb elevations (t(11)=1.29,p=0.22), number of forelimb returns to the floor (t(11)=0.51,p=0.607), number of rears (t(11)=1.95,p=0.08), number of lands (t(11)=−1.79, p=0.1), and number of rears plus lands (t(11)=1.88,p=0.09) did not differ by saline and reboxetine.

4: Discussion

Vocalization and anxiety share several neural substrates. Activations of shared brainstem nuclei, such as the nucleus ambiguus, dorsal motor nucleus of the vagus and nucleus of the solitary tract, and higher brain regions, such as the amygdala and cingulate cortex, are required for both vocalization and autonomic arousal (a physiologic consequence of anxiety). Further, these neural substrates are strongly influenced by monoaminergic systems, including NE, which are greatly disrupted in PD[39]. In the current study, we demonstrate that systemic manipulation of norepinephrine results in changes to both vocalization and anxiety without changing spontaneous motor activity. This is consistent with previous literature that has shown that locomotor activity is not consistently influenced by atomoxetine, reboxetine, or propranolol[42,78,99–101]. The influence of noradrenergic manipulation on vocalization and anxiety, however, is non-uniform.

Administration of atomoxetine resulted in increases in vocal intensity. This is relevant to the *Pink1*−/− rat model of PD, which demonstrate reduced call intensity compared to WT controls [61,63,65]. Further, the increase in vocalization intensity is translationally important to clinical research focused on improving vocal deficits in human PD: vocal intensity is almost universally reduced in human PD, and contributes substantially to communication impairment [102–104]. An additional characteristic of *Pink1*−/− rat vocalization that is altered in comparison to WT controls is that of reduced average peak frequency [61,63,65]. Whereas intensity was increased with atomoxetine in the current study (*i.e.* moved in the direction of WT intensity), principal frequency of calls decreased with atomoxetine (*i.e.* moved away from the direction of WT average peak frequency). While principal frequency (the mean frequency of the spectral contour of the call) and average peak frequency (the frequency at the point in the call with greatest intensity) are not identical measures, they both reflect the central tendency of the frequency of the call. Further, sinuosity of the calls

(a measure of complexity) was reduced with atomoxetine. Ethologically, this change may not be beneficial, as calls with greater average peak frequency and greater complexity have been shown to be important factors in eliciting conspecific approach behavior [84]. Thus, administration of atomoxetine does not “normalize” all aspects of ultrasonic vocalization uniformly. The clinical relevance of changes to average peak frequency and sinuosity, however, is unclear.

While both atomoxetine and a similar norepinephrine reuptake inhibitor, reboxetine, reduced anxiety-like behavior compared to saline, these drugs influenced vocalization differently. Reboxetine reduced the total number of USVs compared to saline, but did not influence acoustic parameter, whereas atomoxetine had an effect on acoustic parameters of vocalization without influencing the total number of USVs. A possible explanation for this finding is that atomoxetine has a higher affinity for serotonin transporter and dopamine transporter [105,106] compared to reboxetine. Dysfunction of several monoaminergic systems is present in PD [27,107,108], and monoaminergic disruption and manipulation are integral to our understanding and therapeutic management of affective disorders for both individuals with and without PD [23,44,109–114]. This disruption is also mirrored in the *Pink1*−/− rat. We have previously observed reduced number of cells stained for tyrosine hydroxylase in the locus coeruleus, and changes to noradrenergic receptors in the nucleus ambiguus and dorsal motor nucleus of the vagus [39,63], as well as reduced immunoreactivity for serotonin in the hypoglossal nucleus (unpublished data). The direct role of these systems and their disruption is less clear in vocalization. Future work that assesses either selective manipulation of other monoamines, or diffuse monoamine manipulation (*i.e.* selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors or serotonin-norepinephrine-dopamine reuptake inhibitors, respectively) would help to clarify this issue.

An unexpected finding of the current study was that administration of the β adrenoreceptor antagonist, propranolol, did not influence vocalization or anxiety in *Pink1*−/−. This contrasts with previous studies of WT rats, which have shown varied changes to ultrasonic vocalization with the same doses of propranolol [42,43]. In previous work (unpublished data), β1 adrenoreceptors in the brainstem have been shown to be altered in *Pink1*−/− rats compared to WT controls. Specifically, the relative optical density of β1 adrenoreceptors was reduced in the nucleus of the solitary tract of *Pink1*−/− rats compared to WT controls, whereas cell count estimates of β1 adrenoreceptor immunoreactive cell bodies in the dorsal motor nucleus of the vagus and the nucleus ambiguus were increased. Further, relative optical density of β1 adrenoreceptors in the nucleus of the solitary tract was significantly positively correlated with call intensity, whereas cell count estimates of β1 adrenoreceptor immunoreactive cells in the dorsal motor nucleus of the vagus and nucleus ambiguus were significantly negatively correlated with call intensity. Thus, a possible explanation for the current findings may be that changes to β1 adrenoreceptor expression in the nucleus of the solitary tract, a complex sensory brainstem nucleus, are a greater driver of vocal deficit than β1 adrenoreceptor disruption in other brain regions. If this were the case, antagonism of β1 adrenoreceptors would not increase vocal intensity, and might even reduce it in the *Pink1*−/− rat. While direct administration of β adrenoreceptor antagonists to lower brainstem nuclei would be beneficial for disentangling the correlational findings reported in this and earlier

works, such direct administration is likely to be challenging due to the relatively small size of these nuclei and their co-localization with cardiac and respiratory neurons.

Because previous work has demonstrated a relationship between anxiety and vocalization in the *Pink1*−/− rat model of PD[39], we conducted exploratory analyses to determine whether this relationship was modulated by systemic manipulation of norepinephrine. None of the drugs administered in this study had an influence on the relationship between anxiety and vocalization. This may have been due to the fact that the study was powered to detect differences in single behavioral measures, rather than behavioral interactions. As a consequence, the sample sizes used in the current study may have been too small to detect relationships between these two outcomes relative to drug condition. This is particularly likely given the variability inherent in animal behavior, which is exchanged for increased genetic and environmental control relative to the study of human patients with PD.

Limitations and Future directions:

A limitation of the current study is that it assessed only immediate changes to vocalization and anxiety following administration of drug doses. Chronic administration of monoamine reuptake inhibitors often results in down-regulation of pre-synaptic receptors, and it is through this longer-term plasticity that changes to behavior are observed [115]. Future studies using this model of PD would benefit from assessing vocalization and anxiety over a longer time course, and assessing changes to neural tissue that might accompany alterations in behavior.

An additional factor that must be considered when interpreting the current findings is that the drug doses used were based on human clinical doses that result in behavioral modification for atomoxetine and propranolol, and on doses that resulted in behavioral changes in WT rats in previous studies for atomoxetine, propranolol, and reboxetine. More extensive dose-response curves for vocalization and anxiety may reveal different effects of the drugs in question, and inclusion of a wild type control group may identify differences in dose-response curves between genotypes. Exploration of additional doses may be particularly relevant for reboxetine, as alterations in dose may reveal the influence of non-selective components of the drug on vocalization or anxiety behavior. This could potentially explain some of the differences observed between atomoxetine and reboxetine. It will be important to complete these dose-response curves with very large samples, as anxiety tests using the plus maze should not be completed less than 3 weeks apart to avoid habituation to the apparatus [70]. The fact that the disease model is progressive complicates repeated testing of anxiety. For example, an animal tested 4 times (at saline, low, medium, high doses) would have 2 doses tested 9 weeks apart. In previous work, we have observed changes in anxiety and vocalization among 2, 4, 6, 8 and 12 month timepoints [62,63]. Thus, very large animal numbers would likely be required for this type of investigation in order to allow for the large degree of variance that is present in animal behavior both by drug dose and by age/disease progression.

Translation of the results from the current study should be tempered by the fact that norepinephrine transporter is similar, but not identical across species in terms of pharmacological properties and distribution [116,117]. As a consequence, effects and

lack of effects of the norepinephrine reuptake inhibitors atomoxetine and reboxetine on vocalization and anxiety observed in the current study may be altered in humans. Prior to completion of prospective, randomized studies assessing the effect of these drugs on vocalization and anxiety in humans with PD, it would be prudent to follow the bi-directional model of translational research suggested by Pankevich and colleagues [118] by completing exploratory investigations assessing vocal function and anxiety in individuals with PD who have and have not been prescribed noradrenergic modulators such as atomoxetine and reboxetine.

Finally, this series of within-subjects experiments is limited in that it does not allow for direct statistical comparison of vocal communication and anxiety across all three drug conditions. Future studies assessing relative efficacy of drugs targeted at modulating vocal communication and anxiety in the *Pink1*−/− rat model of PD would benefit from between-subjects comparisons across drug conditions and saline controls.

Conclusion

Systemic manipulation of norepinephrine results in non-uniform changes to vocalization and anxiety-like behavior in the *Pink1*−/− rat model of PD. Reductions in anxiety (with reboxetine and atomoxetine) and increases in vocal intensity (with atomoxetine) are promising as potential interventions for addressing non-hallmark deficits in PD. A deeper understanding of normal and pathologic interactions among monoaminergic systems is necessary for successful translation of these findings to treatment of vocal communication deficits and anxiety in humans with PD.

Funding:

This work was supported by the National Institutes of Health [NIDCD T32 DC009401; NIDCD, R01 DC014358; NIDCD R01 DC018584-01A1]

Abbreviations:

NE	Norepinephrine
PD	Parkinson Disease
***Pink1*−/−**	*Pink*1 gene knockout
USV	ultrasonic vocalization
WT	wild type

References

[1]. Logemann JA, Fisher HB, Boshes B, Blonsky ER, Frequency and Coocurrence of Vocal Tract Dysfunctions in the Speech of a Large Sample of parkinson Patients, J. Speech Hear. Disord. (1978) 47–57. [PubMed: 633872]

[2]. Sapir S, Ramig L, Fox C, Speech and swallowing disorders in Parkinson disease, Curr. Opin. Otolaryngol. Head Neck Surg. 16 (2008) 205–210. [PubMed: 18475072]

[3]. Huber JE, Darling M, Effect of Parkinson's Disease on the Production of Structured and Unstructured Speaking Tasks: Respiratory Physiologic and Linguistic Considerations, J. Speech, Lang. Hear. Res. 54 (2011) 33–46. [PubMed: 20844256]

[4]. Fox CM, Ramig LO, Vocal Sound Pressure Level and Self-Perception of Speech and Voice in Men and Women With Idiopathic Parkinson Disease, Am. J. Speech-Language Pathol. 6 (1997) 85–94.

[5]. Anand S, Stepp CE, Listener Perception of Monopitch, Naturalness, and Intelligibility for Speakers With Parkinson ' s Disease, J. Speech, Lang. Hear. Res. 58 (2015) 1134–1144. 10.1044/2015. [PubMed: 26102242]

[6]. Matheron D, Stathopoulos ET, Huber JE, Sussman JE, Laryngeal Aerodynamics in Healthy Older Adults and Adults With Parkinson ' s Disease, J. Speech, Lang. Hear. Res. 60 (2017) 507–524. [PubMed: 28241225]

[7]. Stepp CE, Relative fundamental frequency during vocal onset and offset in older speakers with and without Parkinson's disease, J. Acoust. Soc. Am. 133 (2013) 1637–1643. [PubMed: 23464033]

[8]. Broen MPG, Narayen NE, Kuijf ML, Dissanayaka NNW, Leentjens AFG, Prevalence of Anxiety in Parkinson's Disease : A Systematic Review and Meta-Analysis, Mov. Disord. 31 (2016) 1125–1133. 10.1002/mds.26643. [PubMed: 27125963]

[9]. Yamanishi T, Tachibana H, Oguru M, Matsui K, Toda K, Okuda B, Oka N, Anxiety and Depression in Patients with Parkinson's Disease, Intern. Med. 52 (2013) 539–545. 10.2169/internalmedicine.52.8617. [PubMed: 23448761]

[10]. Braak RH, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K, Stages in the development of Parkinson's disease-related pathology, Cell Tissue Res. 318 (2004) 121–134. 10.1007/s00441-004-0956-9. [PubMed: 15338272]

[11]. Bezard E, Fernagut P, Premotor parkinsonism models, Park. Realt. Disord. 20S1 (2014) S17–S19. 10.1016/S1353-8020(13)70007-5.

[12]. Pont-sunyer C, Hotter A, Gaig C, Seppi K, The Onset of Nonmotor Symptoms in Parkinson's Disease (The ONSET PD Study), Mov. Disord. 30 (2015) 229–237. 10.1002/mds.26077. [PubMed: 25449044]

[13]. Hartelius L, Svensson P, Speech and Swallowing Symptoms Associated with Parkinson's Disease and Multiple Sclerosis: A Survey, Folia Phoniatr. Logop. 46 (1994) 9–17. [PubMed: 8162135]

[14]. Midi I, Dogan M, Koseoglu M, Can G, Sehitoglu M, Gunal DI, Voice abnormalities and their relation with motor dysfunction in Parkinson's disease, Acta Neurol. Scand. 117 (2008) 26–34. 10.1111/j.1600-0404.2007.00965.x. [PubMed: 18031561]

[15]. Dissanayaka NNW, Sellbach A, Matheson S, Sullivan JDO, Silburn PA, Byrne GJ, Marsh R, Mellick GD, Anxiety Disorders in Parkinson's Disease: Prevalence and Risk Factors, Mov. Disord. 25 (2010) 838–845. 10.1002/mds.22833. [PubMed: 20461800]

[16]. Pinho P, Monteiro L, Soares FMDP, Tourinho L, Melo A, Nobrega AC, Impact of levodopa treatment in the voice pattern of Parkinson ' s disease patients : a systematic review and meta-analysis O impacto do tratamento com levodopa na, CoDAS. 30 (2018) 1–7. 10.1590/2317-1782/20182017200.

[17]. Rusz J, Tykalová T, Klempíř J, Čmejla R, Růžička E, Effects of dopaminergic replacement therapy on motor speech disorders in Parkinson's disease: longitudinal follow-up study on previously untreated patients, J. Neural Transm. 123 (2016) 379–387. 10.1007/s00702-016-1515-8. [PubMed: 26843071]

[18]. Sanabria J, Ruiz PG, Gutierrez R, Marquez F, Escobar P, Gentil M, Cenjor C, The Effect of Levodopa on Vocal Function in Parkinson's Disease, Clin. Neuropharmacol 24 (2001) 99–102. [PubMed: 11307045]

[19]. Wolfe VI, Garvin JS, Bacon M, Waldrop W, SPEECH CHANGES IN PARKINSON'S DISEASE DURING TREATMENT WITH L-DOPA, J. Commun. Disord 8 (1975) 271–279. [PubMed: 802977]

[20]. Brabenec L, Mekyska J, Galaz Z, Rektorova I, Speech disorders in Parkinson's disease: early diagnostics and effects of medication and brain stimulation, J. Neural Transm. 124 (2017) 303–334. 10.1007/s00702-017-1676-0. [PubMed: 28101650]

[21]. Plowman EK, Kleim JA, Behavioral and neurophysiological correlates of striatal dopamine depletion: A rodent model of Parkinson's disease, J. Commun. Disord. 44 (2011) 549–556. 10.1016/j.jcomdis.2011.04.008. [PubMed: 21601869]

[22]. Ramig L, Halpern A, Spielman J, Fox C, Freeman K, Speech Treatment in Parkinson's Disease: Randomized Controlled Trial (RCT), Mov. Disord. 33 (2018) 1777–1791. 10.1002/mds.27460. [PubMed: 30264896]

[23]. Renfroe JB, Turner TH, Hinson VK, Prevalence, impact, and management of depression and anxiety in patients with Parkinson ' s disease, J. Park. Restless Legs Syndr. 6 (2016) 15–22.

[24]. Calleo JS, Amspoker AB, Sarwar AI, Kunik ME, Jankovic J, Marsh L, York M, Stanley MA, A Pilot Study of a Cognitive–Behavioral Treatment for Anxiety and Depression in Patients With Parkinson Disease, J. Geriatr. Psychiatry Neurol 28 (2015) 210–217. 10.1177/0891988715588831. [PubMed: 26047635]

[25]. Couto MI, Monteiro A, Oliveira A, Lunet N, Massano J, Depression and anxiety following Deep brain stimulation in Parkinson's disease: Systematic review and meta-analysis, Acta Med. Port 27 (2014) 372–382. 10.20344/amp.4928. [PubMed: 25017350]

[26]. Skodda S, Effect of Deep Brain Stimulation on Speech Performance in Parkinson's Disease, Parkinsons. Dis 2012 (2012) 1–10. 10.1155/2012/850596.

[27]. Buddhala C, Loftin SK, Kuley BM, Cairns NJ, Meghan C, Perlmutter JS, Kotzbauer PT, Dopaminergic, serotonergic, and noradrenergic deficits in Parkinson disease, Ann. Clin. Transl. Neurol. 2 (2015) 949–959. 10.1002/acn3.246. [PubMed: 26478895]

[28]. Espay AJ, Lewitt PA, Kaufmann H, Norepinephrine Deficiency in Parkinson's Disease: The Case for Noradrenergic Enhancement Norepinephrine, Mov. Disord. 29 (2014) 1710–1719. 10.1002/mds.26048. [PubMed: 25297066]

[29]. Lewitt PA, Norepinephrine: the next therapeutics frontier for Parkinson's disease, Transl. Neurodegener. 1 (2012) 1–4. [PubMed: 23211032]

[30]. Marien MR, Colpaert FC, Rosenquist AC, Noradrenergic mechanisms in neurodegenerative diseases: a theory, Brain Res. Rev. 45 (2004) 38–78. 10.1016/j.brainresrev.2004.02.002. [PubMed: 15063099]

[31]. Rommelfanger KS, Weinshenker D, Norepinephrine : The redheaded stepchild of Parkinson's disease, Biochem. Pharmacol. 74 (2007) 177–190. 10.1016/j.bcp.2007.01.036. [PubMed: 17416354]

[32]. Del Tredici K, Braak H, Dysfunction of the locus coeruleus – norepinephrine system and related circuitry in Parkinson's disease-related dementia, J. Neurol. Neurosurg. Psychiatry. 84 (2013) 774–783. 10.1136/jnnp-2011-301817. [PubMed: 23064099]

[33]. Vazey EM, Aston-Jones G, The emerging role of norepinephrine in cognitive dysfunctions of Parkinson's disease, Front. Behav. Neurosci. 6 (2012) 1–6. 10.3389/fnbeh.2012.00048. [PubMed: 22279431]

[34]. Cash R, Dennis T, L'Heureux R, Raisman R, Javoy-Agid F, Scatton B, Parkinson's disease and dementia: norepinephrine and dopamine in locus ceruleus., Neurology. 37 (1987) 42–44. [PubMed: 3796837]

[35]. Kreiner G, Rafa-za K, Barut J, Chmielarz P, Kot M, Bagiń M, Parlato R, Daniel WA, Nalepa I, Stimulation of noradrenergic transmission by reboxetine is beneficial for a mouse model of progressive parkinsonism, Sci. Rep 9 (2019) 1–9. 10.1038/s41598-019-41756-3. [PubMed: 30626917]

[36]. Benarroch EE, The locus ceruleus norepinephrine system: Functional organization and potential clinical significance, Neurology. 73 (2009) 1699–1704. 10.1212/WNL.0b013e3181c2937c. [PubMed: 19917994]

[37]. Berridge CW, Waterhouse BD, The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes, Brain Res. Rev. 42 (2003) 33–84. [PubMed: 12668290]

[38]. Dissanayaka NNNW, White E, O'Sullivan JD, Marsh R, Pachana NA, Byrne GJ, The clinical spectrum of anxiety in Parkinson's disease, Mov. Disord. 29 (2014) 967–975. 10.1002/mds.25937. [PubMed: 25043800]

[39]. Hoffmeister JD, Kelm-Nelson CA, Ciucci MR, Quantification of Brainstem Norepinephrine Relative to Vocal Impairment and Anxiety in the Pink1−/− Rat Model of Parkinson Disease, Behav. Brain Res. 414 (2021) 113514. 10.1016/j.bbr.2021.113514. [PubMed: 34358571]

[40]. Marsh L, Biglan K, Gerstenhaber M, Williams J, Atomoxetine for the treatment of executive dysfunction in Parkinson's disease: a pilot open-label study, Mov. Disord. 24 (2009) 277–282. 10.1002/mds.22307.Atomoxetine. [PubMed: 19025777]

[41]. Jankovic J, Atomoxetine for freezing of gait in Parkinson disease, J. Neurol. Sci. 284 (2009) 177–178. 10.1016/j.jns.2009.03.022. [PubMed: 19361809]

[42]. Grant LM, Barth KJ, Muslu C, Kelm-nelson CA, Bakshi VP, Ciucci MR, Noradrenergic Receptor Modulation Influences the Acoustic Parameters of Pro-Social Rat Ultrasonic Vocalizations, Behav. Neurosci 132 (2018) 269–283. [PubMed: 29985007]

[43]. 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. 37 (2012) 808–821. 10.1038/npp.2011.258. [PubMed: 22030713]

[44]. Weintraub D, Mavandadi S, Mamikonyan E, Siderowf AD, Duda JE, Hurtig HI, Colcher A, Horn SS, Navem S, Ten Have TR, Stern MB, Atomoxetine for depression and other neuropsychiatric symptoms in Parkinson disease, Neurology. 75 (2010) 448–455. [PubMed: 20679638]

[45]. Snircova E, Marcincakova-husarova V, Hrtanek I, Kulhan T, Ondrejka I, Nosalova G, Anxiety reduction on atomoxetine and methylphenidate medication in children with ADHD, Pediatr. Int. 58 (2016) 476–481. 10.1111/ped.12847. [PubMed: 26579704]

[46]. Stahl S, Mendel J, Schwartz GE, Effects of Reboxetine on Anxiety, Agitation, and Insomnia : Results of a Pooled Evaluation of Randomized, J. Clin. Psychopharmacol 22 (2002) 388–392. 10.1097/01.jcp.0000024574.36017.14. [PubMed: 12172338]

[47]. Dannon PN, Iancu I, Grunhaus L, The efficacy of reboxetine in the treatment-refractory patients with panic disorder: an open label study, Hum. Psychopharmacol. Clin. Exp. 17 (2002) 329–333.

[48]. Wheatley D, Comparative effects of propranolol and chlordiazepoxide in anxiety states., Br. J. Psychiatry. 115 (1969) 1411–1412. 10.1192/bjp.115.529.1411. [PubMed: 4902187]

[49]. Schank JR, Liles LC, Weinshenker D, Norepinephrine Signaling Through B-Adrenergic Receptors is Critical for Expression of Cocain-Induced Anxiety, Biol. Psychiatry. 63 (2008) 1007–1012. 10.1016/j.biopsych.2007.10.018. [PubMed: 18083142]

[50]. Robinson S, Christ CC, Cahill MM, Aldrich SJ, Taylor-Yeremeeva E, Voluntary exercise or systemic propranolol ameliorates stress-related maladaptive behaviors in female rats, Physiol. Behav. 198 (2019) 120–133. 10.1016/j.physbeh.2018.10.012. [PubMed: 30336229]

[51]. Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF, β-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat, J. Neurosci. 31 (2011) 6277–6288. 10.1523/JNEUROSCI.0450-11.2011. [PubMed: 21525267]

[52]. Steenen SA, Van Wijk AJ, Van Der Heijden GJMG, Van Westrhenen R, De Lange J, De Jongh A, Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis, J. Psychopharmacol 30 (2016) 128–139. 10.1177/0269881115612236. [PubMed: 26487439]

[53]. Meibach RC, Dunner D, Wilson LG, Ishiki D, Dager SR, Comparative efficacy of propranolol, chlordiazepoxide, and placebo in the treatment of anxiety: A double-blind trial., J. Clin. Psychiatry. 48 (1987) 355–358. [PubMed: 3305488]

[54]. Head A, Kendal MJ, Ferner R, Eagles C, Acute effects of β blockade and exercise on mood and anxiety, Br. J. Sports Med. 30 (1996) 238–242. 10.1136/bjsm.30.3.238. [PubMed: 8889119]

[55]. Mealy K, Ngeh N, Gillen P, Fitzpatrick G, Keane FB, Tanner A, Propranolol reduces the anxiety associated with day case surgery, Eur. J. Surg. Acta Chir 162 (1996) 11–14.

[56]. Giddens CL, Barron KW, Clark KF, Warde WD, Beta-Adrenergic Blockade and Voice : A Double-Blind, Placebo-Controlled Trial, J. Voice 24 (2010) 477–489. 10.1016/j.jvoice.2008.12.002. [PubMed: 19846273]

[57]. Carpentier AF, Bonnet AM, Vidailhet M, Agid Y, Improvement of levodopa-induced dyskinesia by propranolol in Parkinson's disease, Neurology. 46 (1996) 1548–1551. 10.1212/WNL.46.6.1548. [PubMed: 8649546]

[58]. Barnum CJ, Bhide N, Lindenbach D, Surrena MA, Goldenberg AA, Tignor S, Klioueva A, Walters H, Bishop C, Effects of noradrenergic denervation on L-DOPA-induced dyskinesia and its treatment by α- And β-adrenergic receptor antagonists in hemiparkinsonian rats, Pharmacol. Biochem. Behav. 100 (2012) 607–615. 10.1016/j.pbb.2011.09.009. [PubMed: 21978941]

[59]. Dave KD, De Silva S, Sheth NP, Ramboz S, Beck MJ, Quang C, Switzer RC, Ahmad SO, Sunkin SM, Walker D, Cui X, Fisher DA, McCoy AM, Gamber K, Ding X, Goldberg MS, Benkovic SA, Haupt M, Baptista MAS, Fiske BK, Sherer TB, Frasier MA, Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease, Neurobiol. Dis. 70 (2014) 190–203. 10.1016/j.nbd.2014.06.009. [PubMed: 24969022]

[60]. Johnson RA, Kelm-Nelson CA, Ciucci MR, Changes to Ventilation, Vocalization, and Thermal Nociception in the Pink1–/–Rat Model of Parkinson's Disease. (Preprint), 1–16., J. Parkinsons. Dis 10 (2020) 489–504. [PubMed: 32065805]

[61]. Kelm-Nelson CA, Yang KM, Ciucci MR, Exercise Effects on Early Vocal Ultrasonic Communication Dysfunction in a PINK1 Knockout MOdel of Parkinson's Disease, J. Park. Dis 5 (2015) 749–763. 10.3233/JPD-150688.

[62]. Marquis JM, Lettenberger SE, Kelm-Nelson CA, Early-onset Parkinsonian behaviors in female Pink1−/− rats, Behav. Brain Res. 377 (2020) 1–15. 10.1016/j.bbr.2019.112175.

[63]. Grant LM, Kelm-Nelson CA, Hilby BL, Blue KV, Rajamanickam ESP, 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. 93 (2015) 1713–1727. 10.1002/jnr.23625. [PubMed: 26234713]

[64]. Dehay B, Bezard E, New Animal Models of Parkinson's Disease, Mov. Disord. 26 (2011) 1198–1205. 10.1002/mds.23546. [PubMed: 22046592]

[65]. Kelm-Nelson CA, Trevino A, Ciucci MR, Quantitative Analysis of Catecholamines in the Pink1 −/− Rat Model of Early-onset Parkinson's Disease, Neuroscience. 379 (2018) 126–141. 10.1016/j.neuroscience.2018.02.027. [PubMed: 29496635]

[66]. Krasko MN, Hoffmeister JD, Schaen-heacock NE, Welsch JM, Kelm-nelson CA, Ciucci MR, Rat Models of Vocal Deficits in Parkinson's Disease, Braiin Sci 11 (2021) 1–21.

[67]. Cullen KP, Grant LM, Kelm-Nelson CA, Brauer AFL, Bickelhaupt LB, Russell JA, Ciucci MR, Pink1 −/− Rats Show Early-Onset Swallowing Deficits and Correlative Brainstem Pathology, Dysphagia. (2018). 10.1007/s00455-018-9896-5.

[68]. Gagnon MA, Langlois Y, Boghen DR, Verdy M, Effects of halazepam and diazepam on the motor coordination of geriatric subjects, Eur. J. Clin. Pharmacol. 11 (1977) 443–448. 10.1007/BF00562936. [PubMed: 19262]

[69]. Robinson ESJ, Eagle DM, Mar AC, Bari A, Banerjee G, Jiang X, Dalley JW, Robbins TW, Similar Effects of the Selective Noradrenaline Reuptake Inhibitor Atomoxetine on Three Distinct Forms of Impulsivity in the Rat, Neuropsychophar. 33 (2008) 1028–1037. 10.1038/sj.npp.1301487.

[70]. Walf AA, Frye CA, The use of the elevated plus maze as an assay of anxiety-related behavior in rodents, Nat. Protoc 2 (2007) 322–328. 10.1038/nprot.2007.44.The. [PubMed: 17406592]

[71]. Adamec R, Shallow T, Effects of baseline anxiety on response to kindling of the right medial amygdala, Physiol. Behav. 70 (2000) 67–80. 10.1016/S0031-9384(00)00247-X. [PubMed: 10978480]

[72]. Adamec R, Shallow T, Burton P, Anxiolytic and anxiogenic effects of kindling - Role of baseline anxiety and anatomical location of the kindling electrode in response to kindling of the right and left basolateral amygdala, Behav. Brain Res. 159 (2005) 73–88. 10.1016/j.bbr.2004.10.004. [PubMed: 15795000]

[73]. Vermoesen K, Massie A, Smolders I, Clinckers R, The antidepressants citalopram and reboxetine reduce seizure frequency in rats with chronic epilepsy, Epilepsia. 53 (2012) 870–878. 10.1111/j.1528-1167.2012.03436.x. [PubMed: 22429158]

[74]. A. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory, Guide for the care and use of laboratory animals, 2011.

[75]. Weintraub D, Mavandadi S, Mamikonyan E, Siderowf A, Duda J, Hurtig H, Colcher A, Horn S, Nazem S, Ten Have T, Stern M, Ten Have TR, Atomoxetine for depression and other

neuropsychiatric symptoms in Parkinson disease, Neurology. 75 (2010) 448–455. [PubMed: 20679638]

[76]. Nair AB, Jacob S, A simple practice guide for dose conversion between animals and human, J. Basic Clin. Pharm. 7 (2016) 27. 10.4103/0976-0105.177703. [PubMed: 27057123]

[77]. Meyerbröker K, Morina N, Emmelkamp PMG, Enhancement of exposure therapy in participants with specific phobia: A randomized controlled trial comparing yohimbine, propranolol and placebo, J. Anxiety Disord. 57 (2018) 48–56. 10.1016/j.janxdis.2018.05.001. [PubMed: 29804894]

[78]. Rygula R, Abumaria N, Havemann-reinecke U, Ruther E, Hiemke C, Zernig G, Fuchs E, Flugge G, Pharmacological validation of a chronic social stress model of depression in rats: effects of reboxetine, haloperidol and diazepam, Behav. Pharmacol. 19 (2008) 183–196. [PubMed: 18469536]

[79]. Connor TJ, Kelliher P, Harkin A, Kelly JP, Leonard BE, Reboxetine attenuates forced swim test-induced behavioural and neurochemical alterations in the rat, Eur. J. Pharmacol. 379 (1999) 125–133. [PubMed: 10497898]

[80]. Pellow S, Chopin P, File SE, Briley M, Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat, J. Neurosci. Methods. 14 (1985) 149–167. 10.1016/0165-0270(85)90031-7. [PubMed: 2864480]

[81]. Hogg S, A review of the validity and variability of the elevated plus- maze as an animal model of anxiety, Pharmacol. Biochem. Behav. 54 (1996) 21–30. [PubMed: 8728535]

[82]. Hopkins ME, Bucci DJ, Interpreting the effects of exercise on fear conditioning: The influence of time of day., Behav. Neurosci 124 (2010) 868–872. 10.1037/a0021200. [PubMed: 21038936]

[83]. Harro J, Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why, Behav. Brain Res. 352 (2018) 81–93. 10.1016/j.bbr.2017.10.016. [PubMed: 29050798]

[84]. Pultorak JD, Kelm-Nelson CA, Holt LR, Blue KV, Ciucci MR, Johnson AM, Decreased approach behavior and nucleus accumbens immediate early gene expression in response to Parkinsonian ultrasonic vocalizations in rats, Soc. Neurosci 11 (2016) 365–379. 10.1080/17470919.2015.1086434. [PubMed: 26313334]

[85]. Bialy M, Rydz M, Kaczmarek L, Precontact 50-kHz vocalizations in male rats during acquisition of sexual experience., Behav. Neurosci 114 (2000) 983–990. 10.1037/0735-7044.114.5.983. [PubMed: 11085613]

[86]. Blanchard RJ, Agullana R, McGee L, Weiss S, Blanchard DC, Sex differences in the incidence and sonographic characteristics of antipredator ultrasonic cries in the laboratory rat (Rattus norvegicus)., J. Comp. Psychol. 106 (1992) 270–277. 10.1037/0735-7036.106.3.270. [PubMed: 1395496]

[87]. Brudzynski SM, Pniak A, Social contacts and production of 50-kHz short ultrasonic calls in adult rats., J. Comp. Psychol. 116 (2002) 73–82. [PubMed: 11926686]

[88]. Riede T, Rat ultrasonic vocalization shows features of a modular behavior, J. Neurosci. 34 (2014) 6874–6878. 10.1523/JNEUROSCI.0262-14.2014. [PubMed: 24828641]

[89]. McGinnis MY, Vakulenko M, Characterization of 50-kHz ultrasonic vocalizations in male and female rats, Physiol. Behav. 80 (2003) 81–88. 10.1016/S0031-9384(03)00227-0. [PubMed: 14568311]

[90]. Wöhr M, Houx B, Schwarting RKW, Spruijt B, Effects of experience and context on 50-kHz vocalizations in rats, Physiol. Behav. 93 (2008) 766–776. 10.1016/J.PHYSBEH.2007.11.031. [PubMed: 18191963]

[91]. Ahrens A, Ma S, Maier E, Duvauchelle C, Schallert T, Robinson T, Repeated Intravenous Amphetamine Exposure: Rapid and Persistent Sensitization of 50-kHz Ultrasonic Trill Calls in Rats, Behav. Brain Res. 197 (2009) 205–209. 10.1016/j.bbr.2008.08.037.Repeated. [PubMed: 18809437]

[92]. Ciucci M, Ma TS, Fox C, Kane J, Ramig L, Schallert T, Qualitative changes in ultrasonic vocalization in rats after unilateral dopamine depletion or haloperidol: A preliminary study, Behav. Brain Res. 182 (2007) 284–289. [PubMed: 17397940]

[93]. Ciucci MR, Ahrens AM, Ma ST, Kane JR, Windham EB, Woodlee MT, Schallert T, Reduction of dopamine synaptic activity: Degredation fo 50-kHz ultrasonic vocalization in rats, Behav. Neurosci 123 (2009) 328–336. 10.1037/a0014593. [PubMed: 19331456]

[94]. Coffey KR, Marx RG, Neumaier JF, DeepSqueak: a deep learning-based system for detection and analysis of ultrasonic vocalizations, Neuropsychopharmacology. 44 (2019) 859–868. 10.1038/s41386-018-0303-6. [PubMed: 30610191]

[95]. Concha-Miranda M, Hartmann K, Reinhold A, Brecht M, Sanguinetti-Scheck JI, Play, but not observing play, engages rat medial prefrontal cortex, Eur. J. Neurosci. 52 (2020) 4127–4138. 10.1111/ejn.14908. [PubMed: 32657503]

[96]. Lenell C, Johnson AM, The effects of the estrous cycle, menopause, and recording condition on female rat ultrasonic vocalizations, Physiol. Behav. 229 (2020) 113248. 10.1016/j.physbeh.2020.113248. [PubMed: 33217390]

[97]. Shembel AC, Lenell C, Chen S, Johnson AM, Effects of Vocal Training on Thyroarytenoid Muscle Neuromuscular Junctions and Myofibers in Young and Older Rats, Journals Gerontol. Med. Sci (2020) 1–9. 10.1093/gerona/glaa173.

[98]. Fleming SM, Zhu C, Fernagut PO, Mehta A, DiCarlo CD, Seaman RL, Chesselet MF, Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone, Exp. Neurol. 187 (2004) 418–429. 10.1016/j.expneurol.2004.01.023. [PubMed: 15144868]

[99]. Turner M, Wilding E, Cassidy E, Dommett EJ, Effects of atomoxetine on locomotor activity and impulsivity in the spontaneously hypertensive rat, Behav. Brain Res. 243 (2013) 28–37. 10.1016/j.bbr.2012.12.025. [PubMed: 23266523]

[100]. Tzavara ET, Bymaster FP, Overshiner CD, Davis RJ, Perry KW, Wolff M, Mckinzie DL, Witkin JM, Nomikos GG, Procholinergic and memory enhancing properties of the selective norepinephrine uptake inhibitor atomoxetine, Mol. Psychiatry. 1 (2006) 187–195. 10.1038/sj.mp.4001763.

[101]. Koda K, Ago Y, Cong Y, Kita Y, Takuma K, Matsuda T, Effects of acute and chronic administration of atomoxetine and methylphenidate on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex and striatum of mice, J. Neurochem. 114 (2010) 259–270. 10.1111/j.1471-4159.2010.06750.x. [PubMed: 20403082]

[102]. Ho AK, Iansek R, Marigliani C, Bradshaw JL, Gates S, Speech impairment in a large sample of patients with Parkinson ‘ s disease, Behav. Neurol. 11 (1998) 131–137. [PubMed: 11568413]

[103]. 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. 24 (2009) 131–44. 10.3233/NRE-2009-0462. [PubMed: 19339752]

[104]. Darley FL, Aronson AE, Brown JR, Clusters of deviant speech dimensions in the dysarthrias., J. Speech Hear. Res. 12 (1969) 462–96. [PubMed: 5811846]

[105]. The Psychoactive Drug Screening Program KI Database, (n.d.).

[106]. Roth BL, Lopez E, Patel S, Kroeze WK, The Multiplicity of Serotonin Receptors: Uselessly Diverse Molecules or an Embarrassment of Riches?, Neurosci. 6 (2000) 252–262. 10.1177/107385840000600408.

[107]. Kano O, Ikeda K, Cridebring D, Takazawa T, Yoshii Y, Iwasaki Y, Neurobiology of Depression and Anxiety in Parkinson’s Disease, Parkinsons. Dis 2011 (2011) 1–5. 10.4061/2011/143547.

[108]. Menza MA, Palermo B, DiPaola R, Sage JI, Ricketts MH, Depression and anxiety in Parkinson’s disease: Possible effect of genetic variation in the serotonin transporter, J. Geriatr. Psychiatry Neurol. 12 (1999) 49–52. 10.1177/089198879901200202. [PubMed: 10483924]

[109]. Slee A, Nazareth I, Bondaronek P, Liu Y, Cheng Z, Freemantle N, Pharmacological treatments for generalised anxiety disorder: a systematic review and network meta-analysis, Lancet. 393 (2019) 768–777. 10.1016/S0140-6736(18)31793-8. [PubMed: 30712879]

[110]. Chaudhuri KR, Healy DG, Schapira AHV, Non-motor symptoms of Parkinson’s disease: diagnosis and management, 5 (2006) 235–245.

[111]. Raskin S, Durst R, Bupropion as the treatment of choice in depression associated with Parkinson's disease and it's various treatments, Med. Hypotheses. 75 (2010) 544–546. 10.1016/j.mehy.2010.07.024. [PubMed: 20708340]

[112]. Williams JW, Mulrow CD, Chiquette E, Noël PH, Aguilar C, Cornell J, A systematic review of newer pharmacotherapies for depression in adults: Evidence report summary, Ann. Intern. Med. 132 (2000) 743–756. 10.7326/0003-4819-132-9-200005020-00011. [PubMed: 10787370]

[113]. Jakubovski E, Johnson JA, Nasir M, Müller-Vahl K, Bloch MH, Systematic review and meta-analysis: Dose–response curve of SSRIs and SNRIs in anxiety disorders, Depress. Anxiety. 36 (2019) 198–212. 10.1002/da.22854. [PubMed: 30479005]

[114]. Schildkraut JJ, The catecholamine hypothesis of affective disorders: a review of supporting evidence., Am. J. Psychiatry. 122 (1965) 509–522. 10.1176/ajp.122.5.509. [PubMed: 5319766]

[115]. Stahl SM, Stahl's essential psychopharmacology: neuroscientific basis and practical applications, 4th ed., Cambridge university press., Cambridge, UK, 2013.

[116]. Smith HR, Beveridge TJR, Porrino LJ, Distribution of norepinephrine transporters in the non-human primate brain, Neuroscience. 138 (2006) 703–714. 10.1016/j.neuroscience.2005.11.033. [PubMed: 16427744]

[117]. Paczkowski FA, Bryan-Lluka LJ, Pörzgen P, Brüss M, Bönisch H, Comparison of the pharmacological properties of cloned rat, human, and bovine norepinephrine transporters, J. Pharmacol. Exp. Ther. 290 (1999) 761–767. [PubMed: 10411589]

[118]. Pankevich DE, Wizemann TM, Altevogt BM, Improving the Utility and Translation of Animal Models for Nervous System Disorders: Workshop Summary, The National Academies Press, Washington, DC, 2013. https://www.ncbi.nlm.nih.gov/books/NBK117243/.

Highlights:

- Atomoxetine and reboxetine reduce anxiety behavior in the *Pink1*−/− rat
- Atomoxetine, but not reboxetine, modulates ultrasonic vocalizations.
- Relationships among anxiety, vocalization and norepinephrine are non-linear

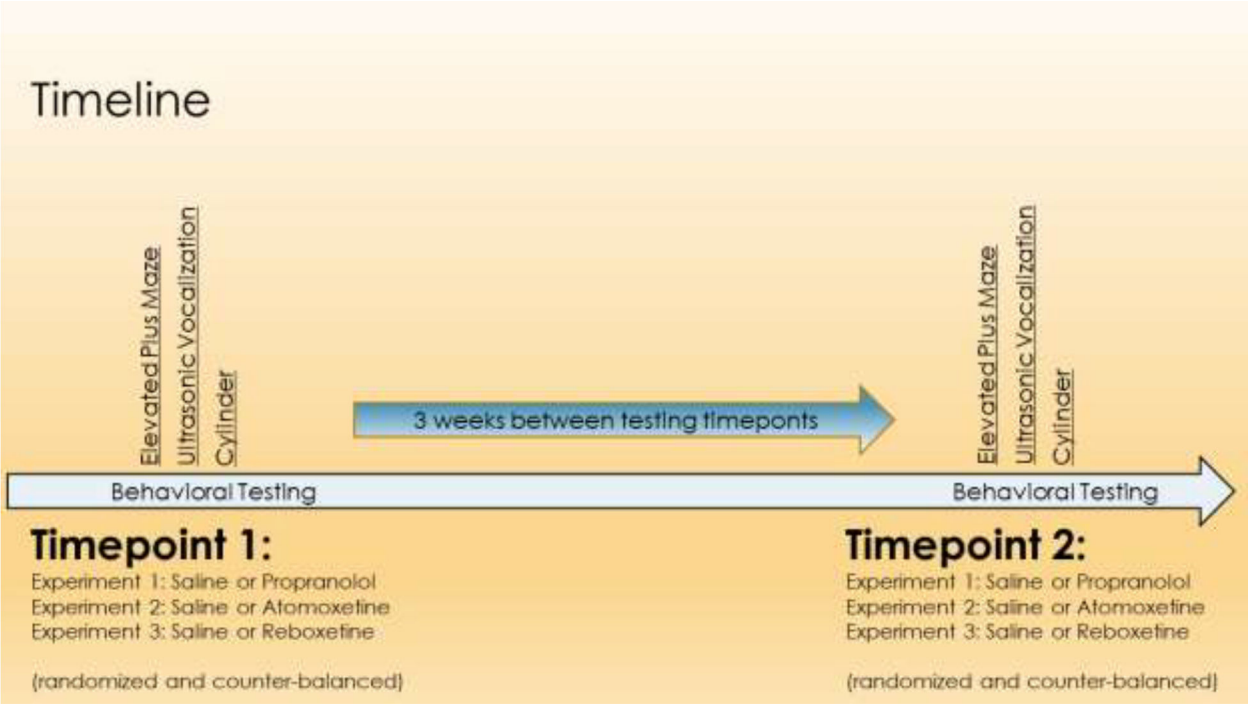

Figure 1: Experimental Timeline

Schematic of experimental timeline. Rats were divided into three experimental groups: propranolol (n at final analysis=12) atomoxetine (n at final analysis=11); reboxetine (n at final analysis=12.) Rats received either saline or drug and underwent behavioral testing at each timepoint. Order of drug versus saline was randomized and counterbalanced in each group.

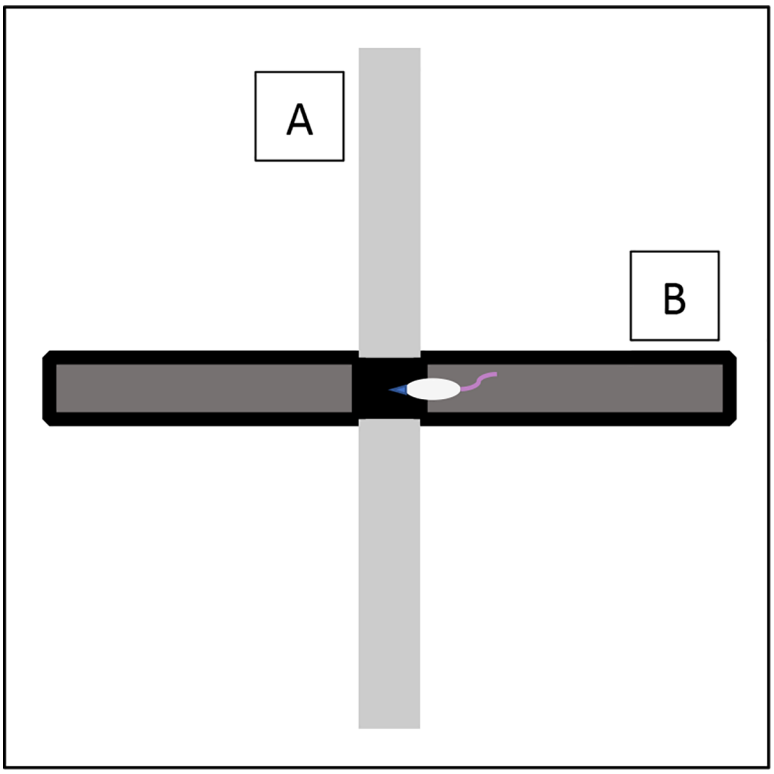

Figure 2: Elevated Plus Maze

Elevated Plus Maze. A: Open Arm; B: Closed Arm. Greater time in closed arms and greater number of entries into closed arms indicates increased anxiety behavior.

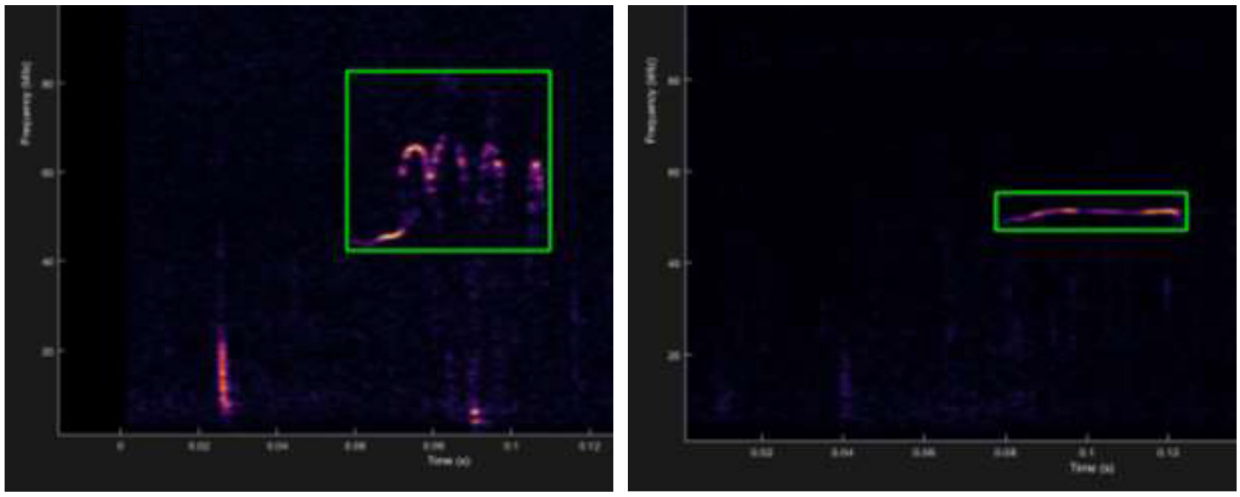

Figure 3: Ultrasonic Vocalization Spectrograms

Examples of spectrograms of ultrasonic vocalizations obtained from DeeqSqueak. Time in seconds is on the x-axis and frequency in kHz is on the y-axis. A: Green box surrounding a complex call; note the frequency modulation between 40 and 65kHz. B: Green box surrounding a simple call; notice the flatness of contour with limited change in frequency around 50kHz.

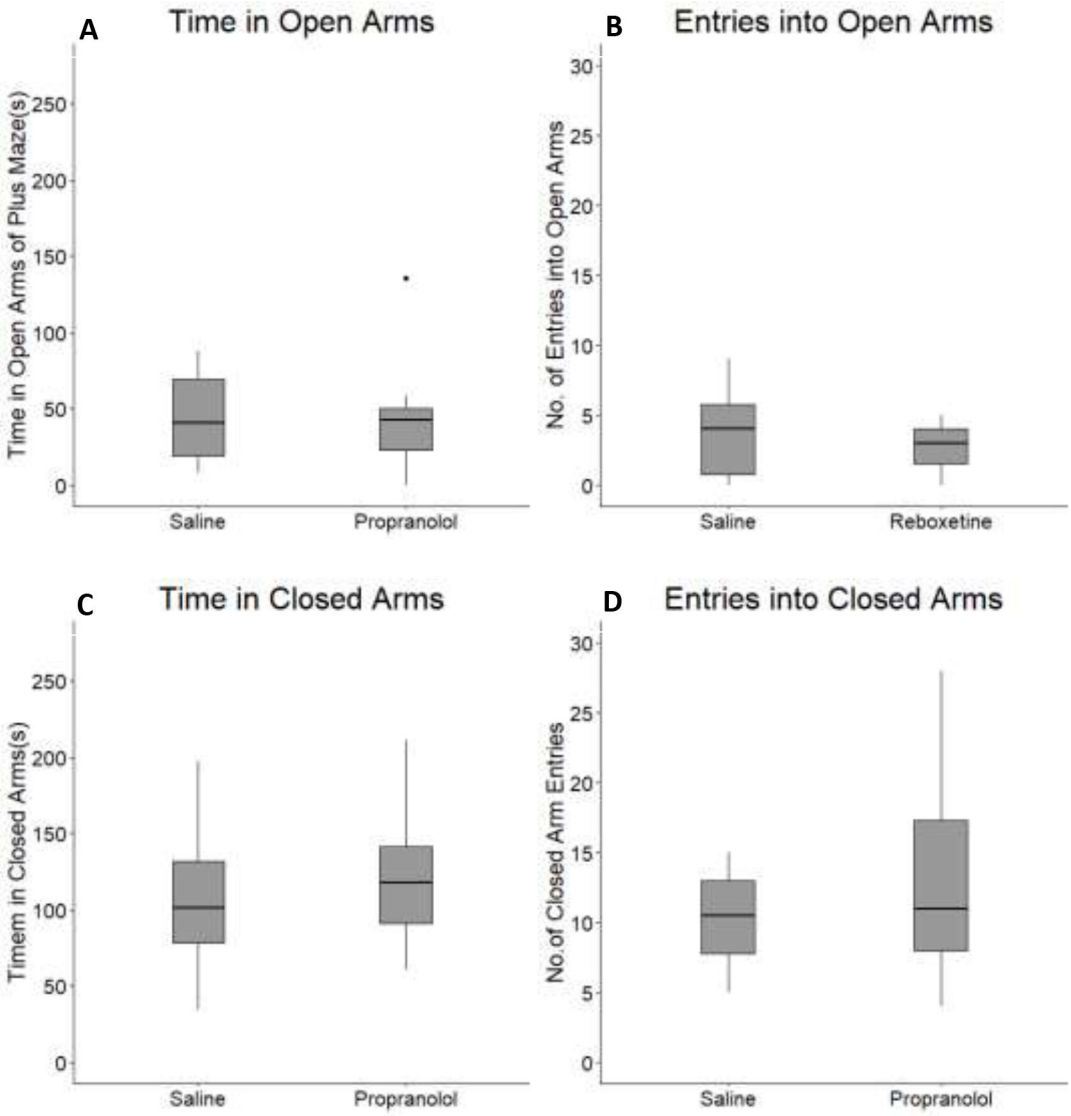

Figure 4:

Anxiety measures comparing propranolol to saline vehicle. **A:** Time spent in open arms of the elevated plus maze (more time in open arms reflects lower anxiety behavior). **B:** Number of entries into open arms (more entries into open arms reflects lower anxiety behavior). **C:** Time spent in closed arms of the elevated plus maze (more time in closed arms reflects higher anxiety behavior). **D:** Number of entries into closed arms (more entries into closed arms indicates higher anxiety behavior). Top and bottom of boxes represent 75^{th} and 25^{th} percentiles respectively; whiskers represent +/−1.5 times the interquartile range; horizontal line through box represents median; dots outside of whiskers represent statistical outliers.

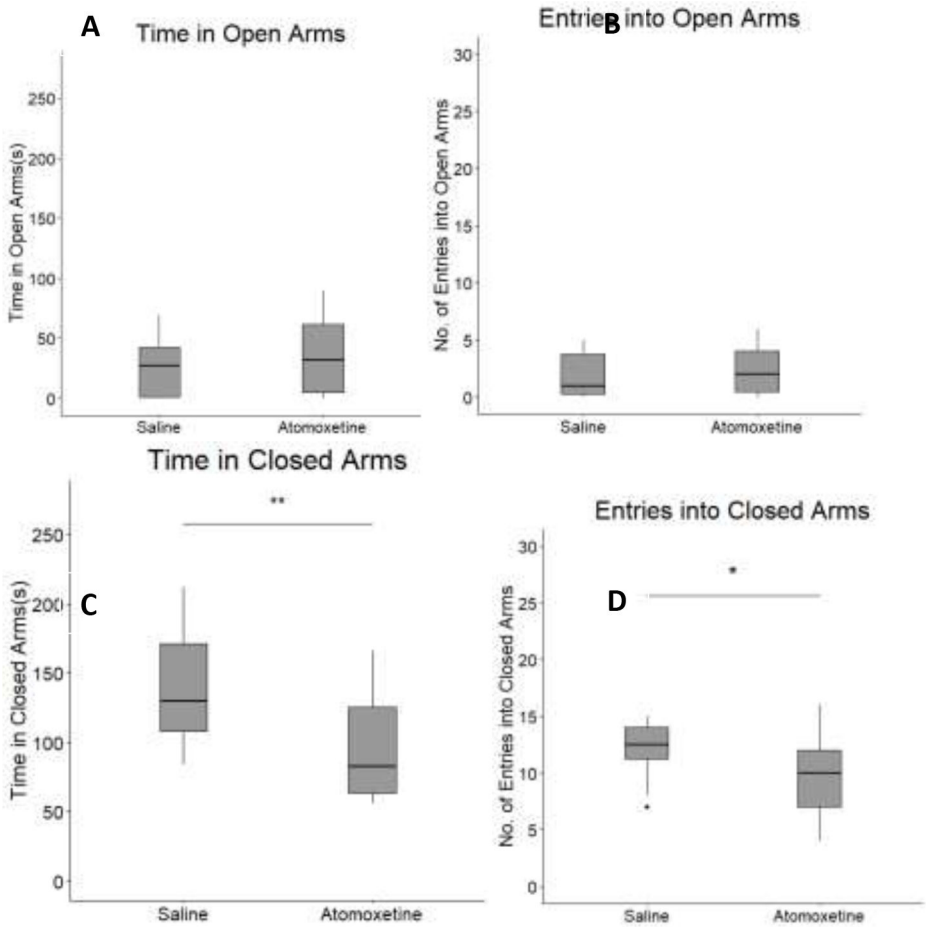

Figure 5:

Anxiety measures comparing atomoxetine to saline vehicle. **A:** Time spent in open arms of the elevated plus maze (more time in open arms reflects lower anxiety behavior). **B:** Number of entries into open arms (more entries into open arms reflects lower anxiety behavior). **C:** Time spent in closed arms of the elevated plus maze (more time in closed arms reflects higher anxiety behavior). **D:** Number of entries into closed arms (more entries into closed arms indicates higher anxiety behavior). Top and bottom of boxes represent 75^{th} and 25^{th} percentiles respectively; whiskers represent +/−1.5 times the interquartile range; horizontal line through box represents median; dots outside of whiskers represent statistical outliers; NS: Non-significant; *: $p<0.05$; **: $p<=0.01$

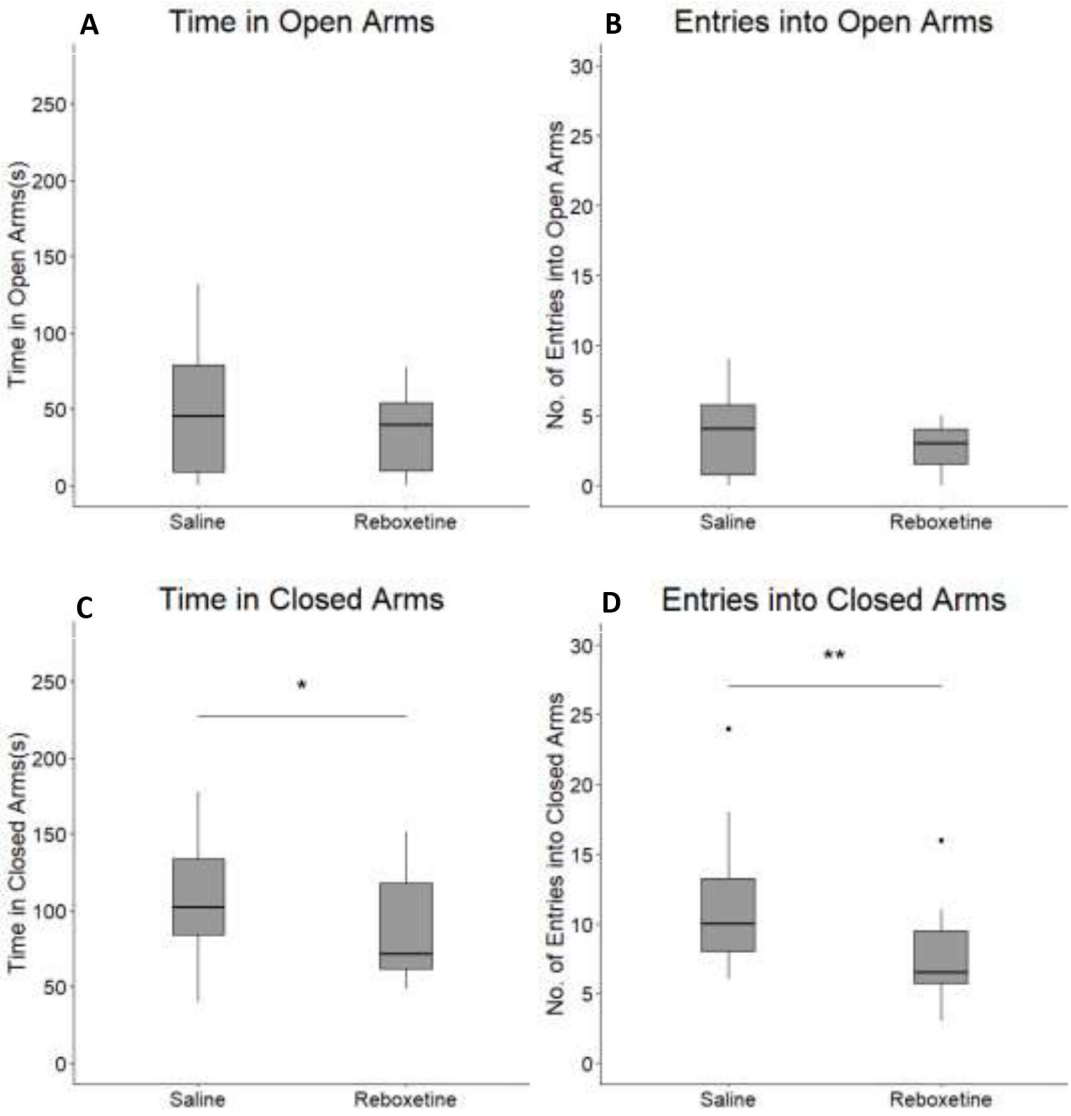

Figure 6:

Anxiety measures comparing reboxetine to saline vehicle. **A:** Time spent in open arms of the elevated plus maze (more time in open arms reflects lower anxiety behavior). **B:** Number of entries into open arms (more entries into open arms reflects lower anxiety behavior). **C:** Time spent in closed arms of the elevated plus maze (more time in closed arms reflects higher anxiety behavior). **D:** Number of entries into closed arms (more entries into closed arms indicates higher anxiety behavior). Top and bottom of boxes represent 75th and 25th percentiles respectively; whiskers represent +/−1.5 times the interquartile range; horizontal line through box represents median; dots outside of whiskers represent statistical outliers; NS: Non-significant; *: $p<0.05$; **: $p<=0.01$.

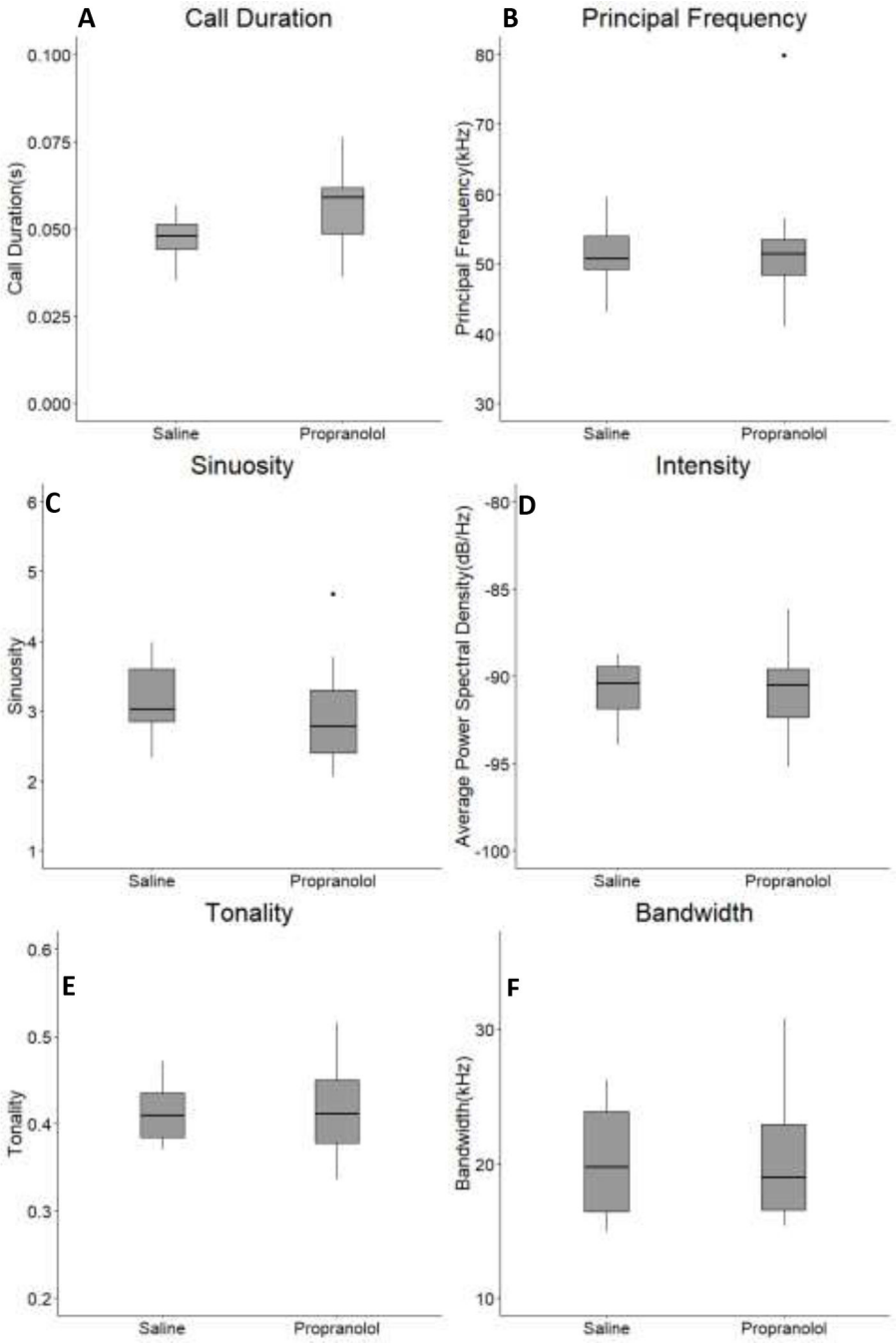

Figure 7:

USV acoustic measures comparing propranolol to saline vehicle for all calls. **A:** Call duration. **B:** Principal frequency (the median frequency along the spectral contour of the call). **C:** Sinuosity (greater sinuosity indicates greater call complexity). **D:** Intensity as measured by average power spectral density in dB/Hz (more-negative indicates reduced loudness). **E:** Tonality as an indication of signal versus noise (greater tonality indicates greater prominence of the signal relative to noise). **F:** Frequency Bandwidth. Top and bottom of boxes represent 75th and 25th percentiles; whiskers represent +/−1.5 times

interquartile range; horizontal line represents median; dots represent outliers; dB: decibels; Hz: hertz.

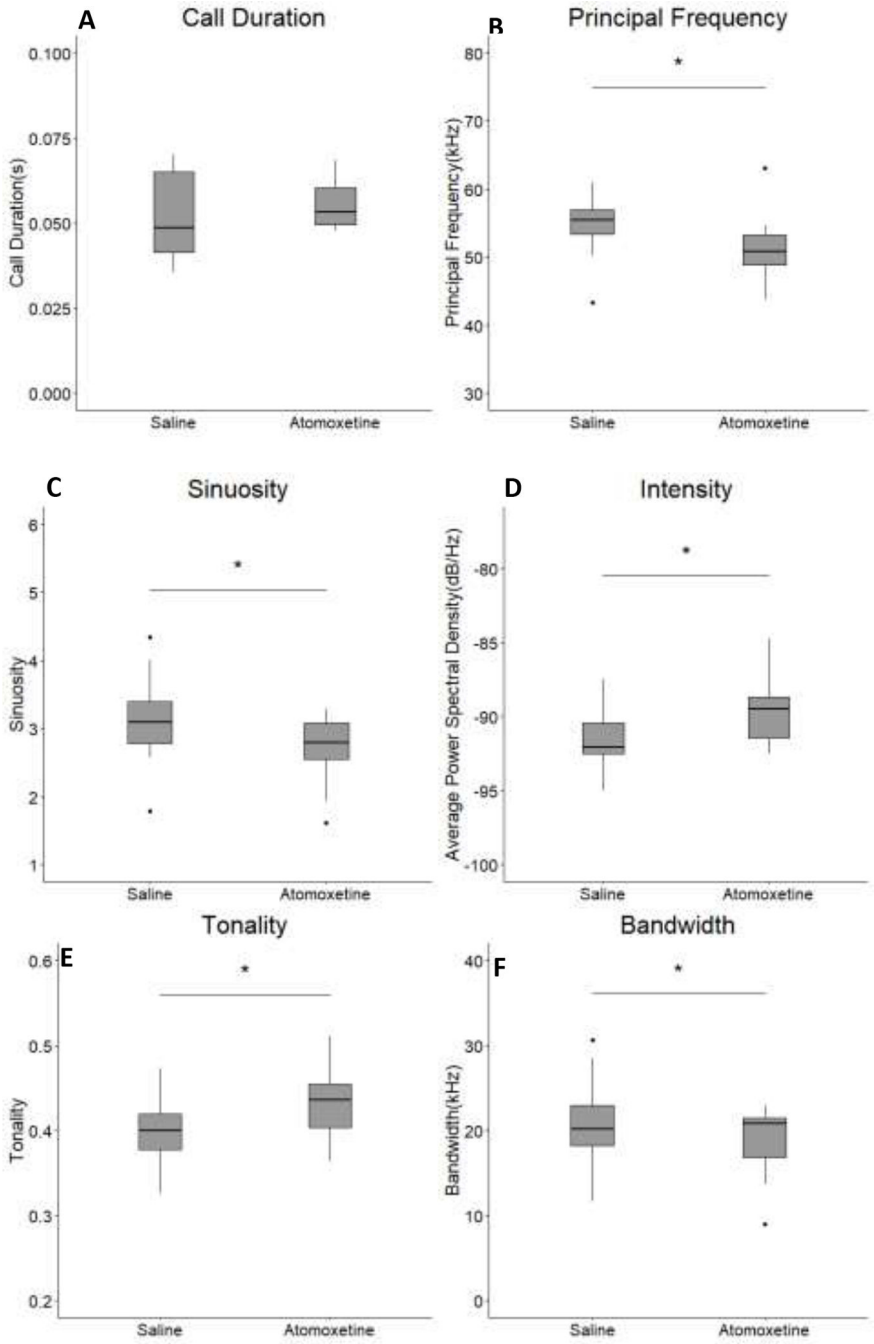

Figure 8:

USV acoustic measures comparing atomoxetine to saline vehicle for all calls. **A:** Call duration. **B:** Principal frequency (the median frequency along the spectral contour of the call). **C:** Sinuosity (greater sinuosity indicates greater call complexity). **D:** Intensity as measured by average power spectral density in dB/Hz (more-negative indicates reduced loudness). **E:** Tonality as an indication of signal versus noise (greater tonality indicates greater prominence of the signal relative to noise). **F:** Frequency Bandwidth. Top and bottom of boxes represent 75th and 25th percentiles; whiskers represent +/−1.5 times

interquartile range; horizontal line represents median; dots represent outliers; *: $p<0.05$; dB: decibels; Hz: hertz.

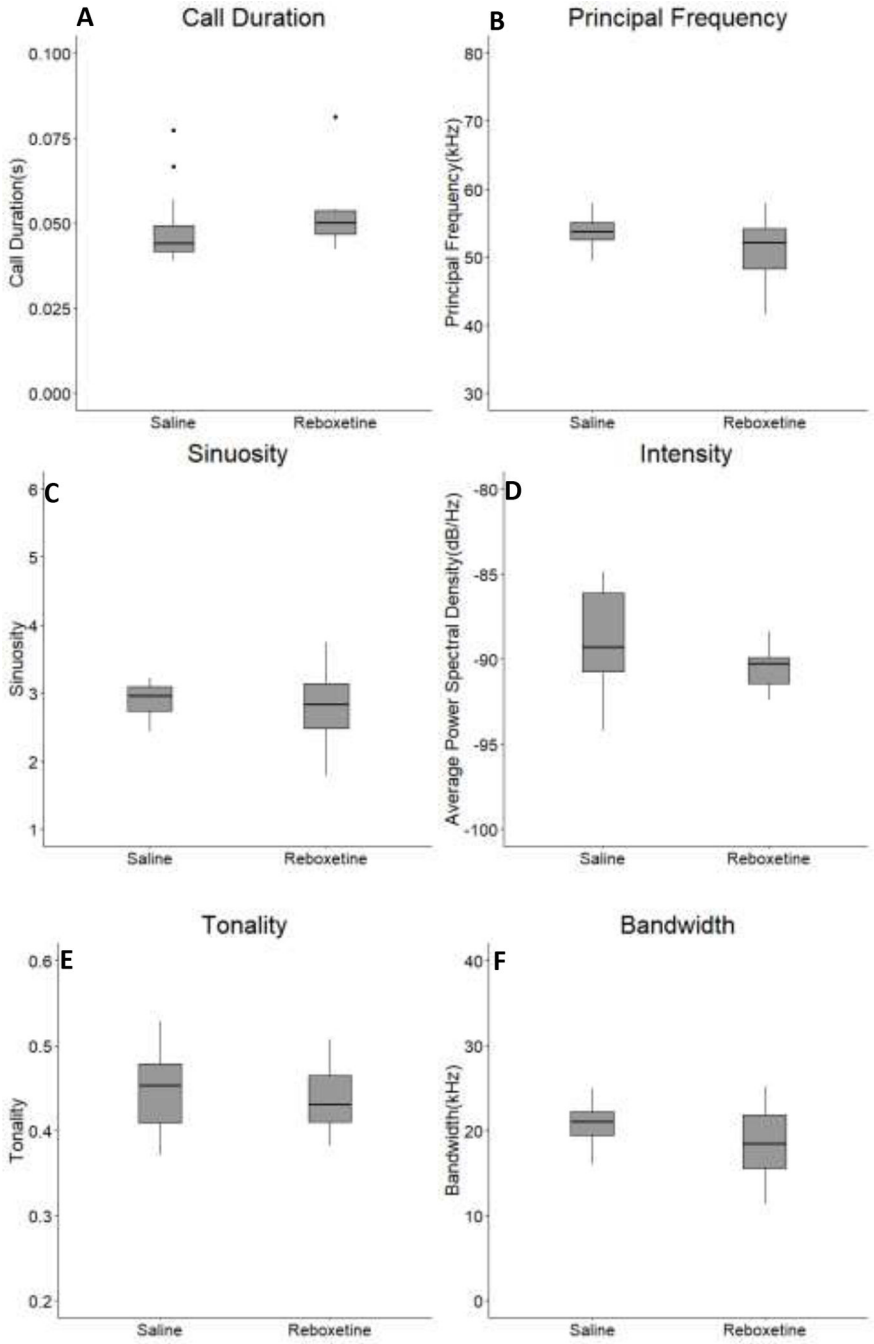

Figure 9:

USV acoustic measures comparing reboxetine to saline vehicle for all calls. **A:** Call duration. **B:** Principal frequency (the median frequency along the spectral contour of the call). **C:** Sinuosity (greater sinuosity indicates greater call complexity). **D:** Intensity as measured by average power spectral density in dB/Hz (more-negative indicates reduced loudness). **E:** Tonality as an indication of signal versus noise (greater tonality indicates greater prominence of the signal relative to noise). **F:** Frequency Bandwidth. Top and bottom of boxes represent 75th and 25th percentiles; whiskers represent +/−1.5 times

interquartile range; horizontal line represents median; dots represent outliers; dB: decibels; Hz: hertz.

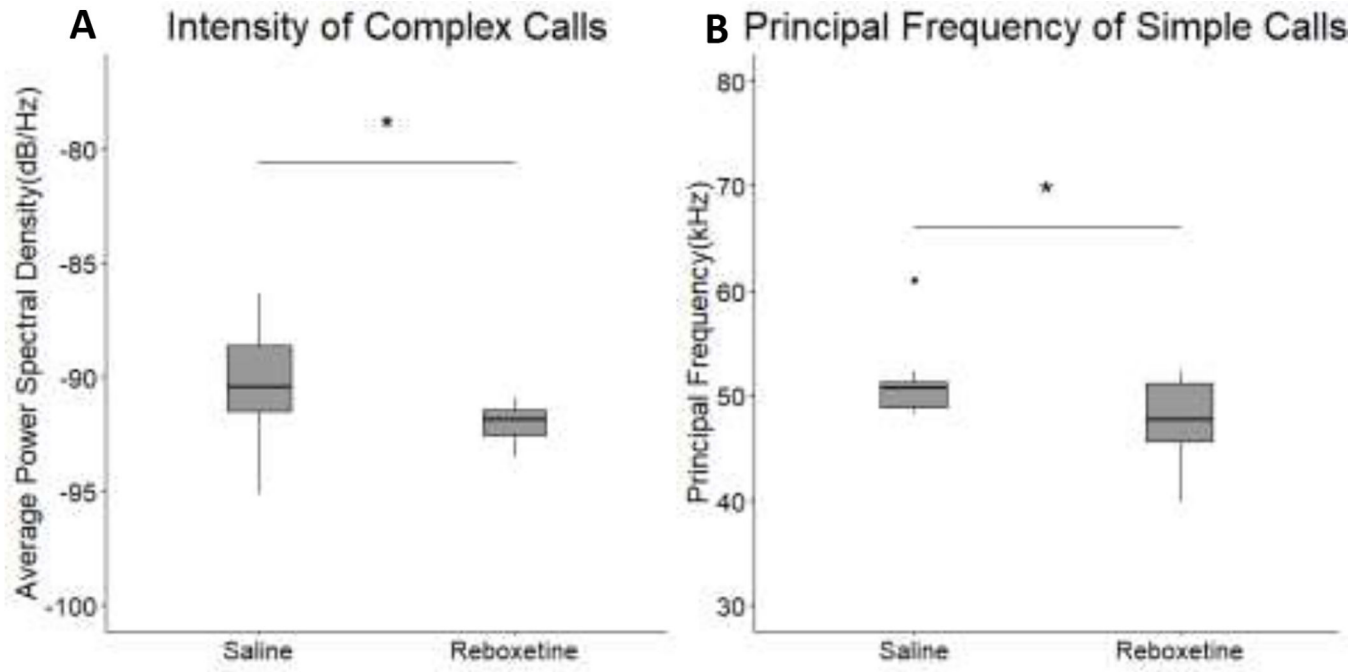

Figure 10:

Comparison of acoustic variables between reboxetine and saline vehicle in sub-categories of simple and complex calls **A:** Intensity of complex calls as measured by average power spectral density in dB/Hz (more-negative indicates reduced loudness). **B:** Principal frequency (the median frequency along the spectral contour of the call) of simple calls. Top and bottom of boxes represent 75th and 25th percentiles respectively; whiskers represent +/−1.5 times the interquartile range; horizontal line through box represents median; dots outside of whiskers represent statistical outliers; *: $p<0.05$; dB: decibels; Hz: hertz

Table 1

Total number of calls and proportion of complex calls to total calls for drug and saline conditions in each of the three experiments

Drug condition	Total number of calls mean (SD)	Proportion of complex calls mean (SD)
Propranolol		
Saline	140.4 (50.97)	0.81(0.14)
Drug	141.4 (50.02)	0.82(0.12)
Atomoxetine		
Saline	115.6 (86.47)	0.81 (0.15)
Drug	119.8 (86.62)	0.78(0.14)
Reboxetine		
Saline	156.1 (40.25)	0.84(0.08)
Drug	103.92 (61.26) *	0.77(0.2)

*: $p < 0.05$ for comparison between saline and drug.