Behavioural Brain Research 307 (2016) 54–64

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Vocal training, levodopa, and environment effects on ultrasonic vocalizations in a rat neurotoxin model of Parkinson disease

Cynthia A. Kelm-Nelson[a,*], Alexander F.L. Brauer[a], Michelle R. Ciucci[a,b,c]

[a] Department of Surgery, Division of Otolaryngology, University of Wisconsin-Madison, Madison, WI 53706, USA
[b] Department of Communication Sciences and Disorders, University of Wisconsin-Madison, Madison, WI 53706, USA
[c] Neuroscience Training Program, University of Wisconsin-Madison, University of Wisconsin-Madison, Madison, WI 53706, USA

HIGHLIGHTS

- Ultrasonic vocalizations are modulated by levodopa and vocal exercise in the unilateral 6-OHDA model.
- Ultrasonic vocalizations and treatments are influenced by testing environments in the unilateral 6-OHDA model.
- Acoustic parameters of ultrasonic vocalizations in the unilateral 6-OHDA model are diminished when tested in a novel versus a familiar environment.

ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form 25 February 2016
Accepted 1 March 2016
Available online 26 March 2016

Keywords:
Parkinson disease
Rat
Ultrasonic vocalization
Levodopa
Vocal exercise
6-OHDA

ABSTRACT

Levodopa does not improve dysarthria in patients with Parkinson Disease (PD), although vocal exercise therapy, such as "LSVT/LOUD®", does improve vocal communication. Most patients receive vocal exercise therapy while concurrently being treated with levodopa, although the interaction between levodopa and vocal exercise therapy on communication in PD is relatively unknown. Further, carryover of vocal exercise therapy to novel situations is critical for successful outcomes, but the influence of novel situations on rehabilitated vocal communication is not well understood. To address the influence of exercise, medications, and environment on vocal communication with precise experimental control, we employed the widely used 6-OHDA rat neurotoxin model of PD (infusion to the medial forebrain bundle), and assessed ultrasonic vocalizations after: vocal exercise, vocal exercise with levodopa, levodopa alone, and control conditions. We tested USVs in the familiar training environment of the home cage and a novel cage. We hypothesized that parkinsonian rats that undergo vocal exercise would demonstrate significant improvement of ultrasonic vocalization (USV) acoustic parameters as compared to the control exercise and levodopa-only treatment groups. We further hypothesized that vocal exercise in combination with levodopa administration, similar to what is common in humans, would lead to improvement in USV outcomes, particularly when tested in a familiar versus a novel environment. We found that the combination of exercise and levodopa lead to some improvement in USV acoustic parameters and these effects were stronger in a familiar vs. a novel environment. Our results suggest that although treatment can improve aspects of communication, environment can influence the benefits of these effects.

Published by Elsevier B.V.

1. Introduction

Parkinson Disease (PD) is a complex neurodegenerative condition primarily attributed to dopamine depletion and the degeneration of the nigrostriatal pathways [1–4], although the pathology of PD is vastly complex [1,5–7]. Sensorimotor deficits have devastating effects on quality of life in people with PD [8]. Voice and speech deficits are common early-onset complications of PD and in humans, clinical speech and voice therapy (exercise), such as LSVT/LOUD®, has been shown to improve vocalizations [9–15]. However, patients must transition and carry over therapy techniques from the clinic to everyday activities in order to have successful speech treatment outcomes and drive long-lasting changes [16]. For example, weak responders to LSVT/LOUD® may require additional or longer training as well as increases in communication during daily activities [17]. Thus, there is a significant gap in our understanding of how the effects of exercise are influ-

* Corresponding author at: Department of Surgery, Division of Otolaryngology, 1300 University Avenue, 483 Medical Sciences Center, University of Wisconsin-Madison, Madison, WI 53706, USA.
E-mail address: CAKelm@wisc.edu (C.A. Kelm-Nelson).

http://dx.doi.org/10.1016/j.bbr.2016.03.006
0166-4328/Published by Elsevier B.V.

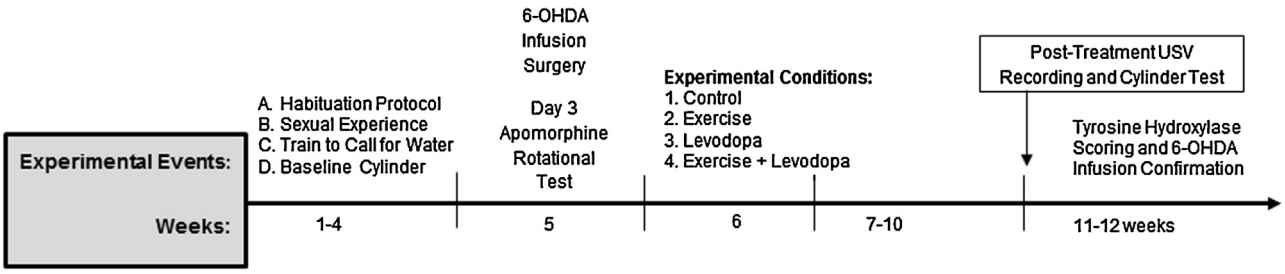

Fig. 1. Experimental timeline in weeks. Overview of the experimental timeline, conditions and testing.

enced by the communication environment. Further, vocal exercise therapy is typically conducted while patients are also being treated with pharmacological methods of dopamine augmentation such as levodopa [15,18]. The effects of therapy, levodopa, and the environment have not been studied with precise experimental control due to the inherent variability associated with studying human patients. Thus, we studied the effects of medication, exercise, and environment in a widely accepted rat model of Parkinsonism: unilateral infusion of the neurotoxin 6-OHDA to the medial forebrain bundle [19–27].

Rodent models are a powerful research tool to study sensorimotor deficits associated with PD [28,29]. Previous studies in rats with unilateral infusion 6-OHDA suggest that targeted exercise may reverse and/or slow disease progression by sparing striatal dopamine and decreasing signs of Parkinsonism [30–32]. For example, forced use of a limb results in behavioral sparing when exercise training is initiated before or early after the administration of a dopamine-depleting neurotoxin [31,33–35]. Chronic levodopa therapy improves some gross motor deficits such as rotational responses [36], but not skilled forelimb movements including reaching and grasping for food [37]. In rats, early exercise intervention (therapy) has been shown to protect viable dopamine neurons in the substantia nigra as well as improve behavioral sensorimotor outcomes [20,26,35,38,39]. However, exercise paradigms are typically carried-out in the drug-free state, and as with humans, and the potential interactions between levodopa and exercise are currently unknown.

Rats are known to communicate through ultrasonic vocalizations (USVs) in a variety of social situations including mating [40–46], and have been used to investigate how PD-related pathology influences vocalizations [20,23,25,47,48]. Rats produce several types of USVs that are classified by frequency and complexity of the waveform. Rats produce social USVs during a mating paradigm that fall within the 50-kilohertz (kHz) range [46] and are composed of short constant frequency calls (simple) or frequency modulated (FM, complex) subtypes [42,44]. These calls are a useful model to study phonatory deficits associated with PD as they parallel human vocal communication in many ways [40,42,44,49]. Recent work has shown that unilateral 6-OHDA infusion to the medial forebrain bundle is sufficient to degrade acoustic parameters of 50-kHz calls (reduced bandwidth, intensity and peak frequency), and that altering dopaminergic synaptic transmission with haloperidol is sufficient to degrade the acoustic signal in rat 50-kHz USVs [25]. Furthermore, rat 50-kHz USVs are influenced by dopaminergic modulation. For example, pharmacological antagonism of dopamine D1 and D2 receptor subtypes results in reductions to call rate and complexity [23,25,50,51]. Together, these studies suggest that rat 50-kHz USVs are susceptible to neurodegenerative processes and modulated to some degree by dopaminergic systems.

The purpose of the present study was to determine the extent of improvement and/or recovery of vocal function in rats with unilateral 6-OHDA infusions after administration of targeted vocal exercise and/or levodopa treatment. We analyzed the acoustic properties of USVs in rats with unilateral infusion of 6-OHDA after 4 weeks of: sham-controls, exercise, levodopa, exercise and levodopa. These effects were measured in the animal's home cage (where they were housed and trained-referred to as a familiar environment) and in a novel cage, to measure effects of environment on 50-kHz USV production. To confirm lesions, striatal dopamine depletion was quantified using a combination of behaviors (cylinder forelimb placing and apomorphine rotations) and histologic measures of tyrosine hydroxylase (TH) immunohistochemistry in the striatum. We hypothesized that intervention using targeted-vocal exercise would improve acoustic parameters in animals with 6-OHDA infusion. We also hypothesized that exercise in combination with levodopa treatment would significantly improve USV production compared to levodopa and exercise alone. Additionally, we hypothesized that USV production would be significantly different (improved) in the familiar home cage versus a novel test cage.

2. Materials and methods

2.1. Animals and habituation

Twenty-four male Long-Evans rats (Charles River Laboratories, Raleigh, NC, USA), aged 3 months at the time of study onset, were housed in single-sex groups of two in standard polycarbonate cages with corncob bedding on a reversed 12:12 h (hr) light: dark cycle. All testing occurred during the dark period of the cycle under red light. Food and water was available ad libitum except during vocalization and control exercise, where water was periodically restricted (see below). Animals were handled daily starting at 2 months of age. Female rats (n = 15) were used to elicit vocalizations from male rats. The female rats were brought into estrous through subcutaneous injections of 10 μg of β-estradiol (Sigma Aldrich, St. Louis, MO, USA) and 500 μg of progesterone (Sigma Aldrich, St. Louis, MO, USA) in sterile vehicle (sesame seed oil; Fisher Scientific, Pittsburgh, PA, USA) at 48 h and 4 h before behavioral testing, respectively. Female rats were not included in the data analysis and were only used for eliciting vocalizations for vocal exercise, control exercise, and testing in familiar and novel cages (see below). All protocols and procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee (IACUC) and were conducted in accordance with the United States Public Health Service Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MA, USA).

2.2. Overview of experimental testing

Refer to Fig. 1. Rats were handled daily by the experimenter, habituated to the recording apparatus and procedures, and were provided with sexual experience with a female conspecific starting at 2 months of age. At 3 months of age, the cylinder test for spontaneous activity was used to assess general motor behavior at baseline. Following baseline testing and habituation, animals