Brain Research 1655 (2017) 233–241

Contents lists available at ScienceDirect

Brain Research

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

Research report

Effects of subthalamic deep brain stimulation with duloxetine on mechanical and thermal thresholds in 6OHDA lesioned rats

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ARTICLE INFO

Keywords:
Subthalamic deep brain stimulation
Parkinson's disease
Mechanical allodynia
Thermal hyperalgesia
Hot plate test
Von Frey test
Randall-Selitto test
Duloxetine

ABSTRACT

Chronic pain is the most common non-motor symptom of Parkinson's disease (PD) and is often overlooked. Unilateral 6-hydroxydopamine (6-OHDA) medial forebrain bundle lesioned rats used as models for PD exhibit decreased sensory thresholds in the left hindpaw. Subthalamic deep brain stimulation (STN DBS) increases mechanical thresholds and offers improvements with chronic pain in PD patients. However, individual responses to STN high frequency stimulation (HFS) in parkinsonian rats vary with 58% showing over 100% improvement, 25% showing 30–55% improvement, and 17% showing no improvement. Here we augment STN DBS by supplementing with a serotonin-norepinephrine reuptake inhibitor commonly prescribed for pain, duloxetine. Duloxetine was administered intraperitoneally (30 mg/kg) in 15 parkinsonian rats unilaterally implanted with STN stimulating electrodes in the lesioned right hemisphere. Sensory thresholds were tested using von Frey, Randall-Selitto and hot-plate tests with or without duloxetine, and stimulation to the STN at HFS (150 Hz), low frequency (LFS, 50 Hz), or off stimulation. With HFS or LFS alone (left paw; $p=0.016$; $p=0.024$, respectively), animals exhibited a higher mechanical thresholds stable in the three days of testing, but not with duloxetine alone (left paw; $p=0.183$). Interestingly, the combination of duloxetine and HFS produced significantly higher mechanical thresholds than duloxetine alone (left paw, $p=0.002$), HFS alone (left paw, $p=0.028$), or baseline levels (left paw; $p < 0.001$). These findings show that duloxetine paired with STN HFS increases mechanical thresholds in 6-OHDA-lesioned animals more than either treatment alone. It is possible that duloxetine augments STN DBS with a central and peripheral additive effect, though a synergistic mechanism has not been excluded.

1. Introduction

Chronic pain is the most common non-motor symptom in Parkinson's disease (PD) (Beiske et al., 2009). Pain can be nociceptive or neuropathic (Truini et al., 2013). Nociceptive pain is most frequent (40–90%) and is typically musculoskeletal and visceral (Wasner and Deuschl, 2012). Significantly lower mechanical, thermal, and chemical thresholds compared to healthy controls have been shown in PD patients (Fil et al., 2013) and animal models of PD (Lin et al., 1981; Saade et al., 1997; Chudler and Lu, 2008; Marques et al., 2013; Zengin-Toktas et al., 2013; Park et al., 2015). Chronic pain is a significant problem leading to PD patients seeking various medical treatments and surgeries that may turn out to be ineffective and unnecessary.

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been found to decrease pain in PD patients (Kim et al., 2008; Zahodne et al., 2009). Specifically, STN DBS has shown to improve pre-operative PD-related pain for up to eight years; however, 18 of 24 (75%) patients developed new pain symptoms over this time period (Jung et al., 2015). We have previously demonstrated both 150 and 50 Hz STN DBS improve mechanical thresholds in 6-hydroxydopamine (6OHDA) lesioned rats (Gee et al., 2015). The response showed overall improvement, with 7 of 12 animals improving over 100% at 150 Hz, 7 of 12 improving over 100% on 50 Hz, and 5 of the 12 animals improving over 100% on both settings compared to baseline Von Frey (VF) mechanical thresholds. Four animals were non-responders, without increased thresholds on either setting, showing that individual rats may respond differently and that there is potential for improving DBS therapy (Gee et al., 2015). Current findings have not fully demonstrated the mechanism of action and ability to consistently provide benefit.

In order to augment the effect of DBS on mechanical and thermal thresholds, this study evaluates the effects of administering STN DBS in combination with duloxetine, a non-narcotic pain medication commonly used in clinical settings. Duloxetine is a serotonin norepinephrine reuptake inhibitor (SNRI) commonly administered for anti-

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http://dx.doi.org/10.1016/j.brainres.2016.10.025
Received 29 April 2016; Received in revised form 24 October 2016; Accepted 27 October 2016
Available online 29 October 2016
0006-8993/

depression, anxiolysis, and anti-hyperalgesia, shown to significantly improve pain in PD patients and thresholds in rat models of neuropathic pain (Djaldetti et al., 2007; Bellingham and Peng, 2010; Vollmer et al., 2014; Wang et al., 2015). It does not cause drowsiness to the extent narcotics, opioids, and gabapentin do and thus may be a better option for PD patients, a population of mostly elderly individuals susceptible to a high fall risk (American Geriatrics Society Panel on the Pharmacological Management of Persistent Pain in Older Persons, 2009). Further, 65% of PD patients reported a positive effect on chronic pain with duloxetine (Djaldetti et al., 2007).

As DBS and some anti-depressants have been found to have augmenting properties when employed together in treatment of other diseases (Hamani and Nobrega, 2012), we assess whether combined therapy of STN DBS with duloxetine increases maximal mechanical and thermal threshold improvement.

2. Results

2.1. Confirmation of 6OHDA lesion and PD phenotype

In order to confirm the PD phenotype in the 6OHDA-lesioned rats (n=15), a behavioral limb asymmetry test (LAT) and tyrosine hydroxylase (TH) quantification of the striatum and SNc were used. Sham rats exhibit approximately 50% right paw touches (Gee et al., 2015) and therefore the 6OHDA lesioned rats had paw touches statistically compared to a theoretical 50% mean value using a one-sample *t*-test (Fig. 1A). Rats showed significantly greater number of right paw touches (94.65% ± 1.90%; $p < 0.001$; t=23.51; DF=14) indicating left limb akinesia (Fig. 1A). Quantification of TH staining for dopamine depletion measurement was conducted using pixel quantification in ImageJ. Typically shams exhibit a 0% depletion of dopamine while 6OHDA lesioned rats often show a greater than 80% depletion (Gee et al., 2015). A comparison of our values to 0% depletion with a one-sample *t*-test revealed that 6OHDA brain hemispheres contained more than 80% average depletion of dopamine compared to the contralateral side in both the striatum (88.16% ± 3.14%; $p < 0.001$; t=28.08; DF=14) and SNc (86.22% ± 3.41%; $p < 0.001$; t=25.29; DF=14) (Fig. 1B). Fig. 1C and D display a 6OHDA lesioned hemisphere in the striatum and SNc, respectively. Cresyl violet staining of the STN region was used to confirm STN DBS electrode placements with respect to the rat atlas (Paxinos and Watson, 1998) (Fig. 2A; B). An additional four rats with misplaced electrodes, and/or failed TH quantifications were excluded. Rats that did not score over 80% on the LAT were excluded and no further behavioral testing was performed.

2.2. Effect of STN DBS on sensory thresholds

Only 6OHDA-lesioned rats (*n*=15) with OFF-stimulation values below cut-off maximum fiber response (< 26.0 g) for VF mechanical testing were used in stimulation/duloxetine experiments. Maximum fiber response was defined as the highest fiber strength applied that did not raise the rat's hindpaw. An additional two were excluded for this reason. In all three days of mechanical testing prior to duloxetine administration, HFS alone showed significant improvement in increasing VF thresholds from baselines at 4.29 g ± 0.60–11.50 g ± 2.00 g (RM-ANOVA, $p_{Day1}=0.009$, $F_{3,42}=5.12$; Fig. 3A), 13.24 g ± 2.06 g (RM-ANOVA, $p_{Day2}=0.001$, $F_{3,42}=5.12$; Fig. 3A), and 13.93 g ± 2.90 g (RM-ANOVA, $p_{Day3}=0.023$, $F_{3,42}=5.12$; Fig. 3A). With LFS alone, significant improvement was seen on day 1 and 2, increasing thresholds from baseline 4.29 g ± 0.60–10.26 g ± 2.24 g (RM-ANOVA, $p_{Day1}=0.043$, $F_{3,42}=3.95$; Fig. 3B), 12.58 g ± 2.62 g (RM-ANOVA, $p_{Day2}=0.024$, $F_{3,42}=3.95$; Fig. 3B), and 11.09 g ± 2.55 g (RM-ANOVA, $p_{Day3}=0.075$, $F_{3,42}=3.95$; Fig. 3B).

2.3. Effect of duloxetine on sensory thresholds

To determine whether 30 mg/kg IP administration of duloxetine alters pain thresholds in the 6OHDA-lesioned rat model, mechanical and thermal pain thresholds at baseline without the drug were compared to thresholds with the drug without stimulation. Duloxetine alone did not significantly raise sensory thresholds above baseline in 6OHDA lesioned animals for mechanical testing but did so with thermal testing.

There was significant increase in thermal HPT thresholds at

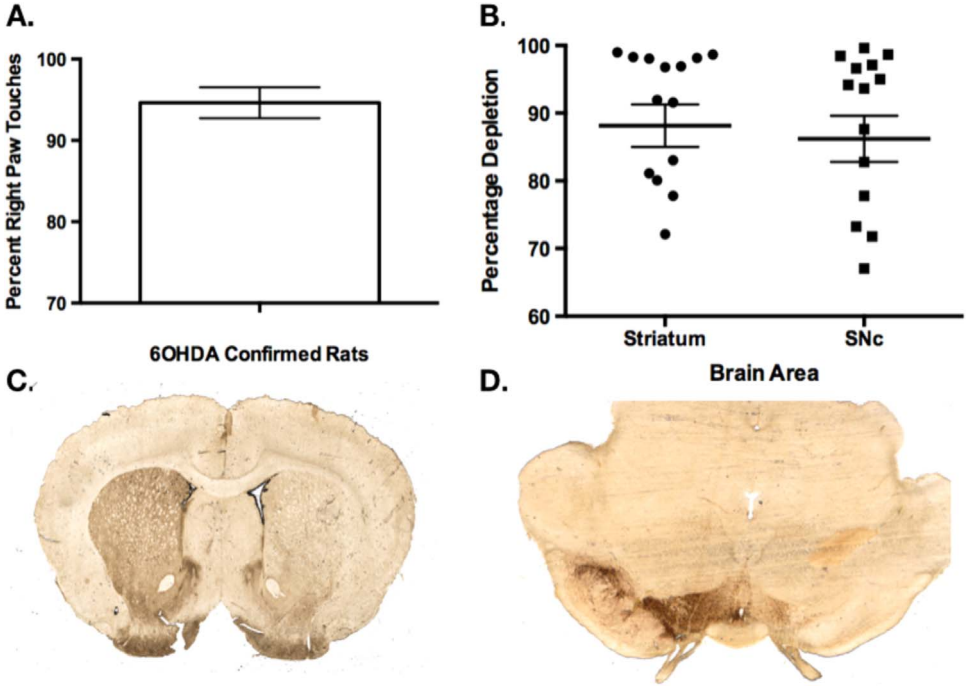

Fig. 1. PD confirmation of 6ODHA rats. (A) 6OHDA-lesioned animals (n=15) exhibited > 80% of right touches (94.65% ± 1.90%). (B) Percent quantification of dopaminergic depletion represented by line bars with standard error of mean (SEM). TH staining in the striatum (C) and SNc (D) reveals dopaminergic cell loss in 6OHDA-lesioned hemisphere, as compared to the contralateral hemisphere.