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Research report

Investigation of diazepam efficacy on anxiety-like behavior in hemiparkinsonian rats

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HIGHLIGHTS

- Hemiparkinsonian rats have higher anxiety-like behavior than non-parkinsonian rats.
- Hemiparkinsonian rats have the greatest prevalence of 'high anxiety' behavior.
- Diazepam is least efficacious in high anxiety hemiparkinsonian rats.

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ABSTRACT

There is growing recognition that anxiety disorders have a greater impact on quality of life in Parkinson's disease than motor symptoms. Yet, little is known about the pathophysiology underlying this non-motor symptom in Parkinson's disease which poses a considerable barrier in developing effective treatment strategies. Here, we administered diazepam to hemiparkinsonian and non-parkinsonian rats and assessed its efficacy in three anxiety behavioral tests. At present, no information about this exists in preclinical research with sparse data in the clinical literature. Moreover, diazepam is an acute anxiolytic which makes this drug a suitable research tool to unmask differences in anxiety-like behavior. Using the unilateral, medial forebrain bundle 6-hydroxydopamine rat model of Parkinson's disease, we noted that hemiparkinsonian rats had more baseline anxiety-like behavior with 60% of them exhibiting high anxiety (HA) behavior in the elevated plus maze. In contrast, 41% of sham-lesioned rats and 8% of naïve rats exhibited HA behavior. Next, we employed the elevated plus maze and noted that diazepam (1.5 mg/kg) was anxiolytic in low anxiety (LA) sham-lesioned ($p = 0.006$) and HA sham-lesioned rats ($p = 0.016$). Interestingly, diazepam was anxiolytic for LA hemiparkinsonian rats ($p = 0.017$), but not for HA hemiparkinsonian rats ($p = 0.174$) despite both groups having similar motor impairment and parkinsonian phenotype. Overall, diazepam administration unmasked differences in anxiolytic efficacy between HA hemiparkinsonian rats, LA hemiparkinsonian rats and non-parkinsonian rats. Our data suggests that neuro-circuits involved in anxiety-like behavior may differ within these groups and posits that diazepam may have reduced efficacy in certain individuals with PD anxiety disorders.

1. Introduction

Clinically significant anxiety is a non-motor symptom in Parkinson's disease (PD) that can affect up to 75% of PD patients [1], although most studies show a prevalence in the 20–40% range. This variability reflects the lack of a standardized PD-specific anxiety test [2], the wide spectrum of anxiety disorders in PD [3] and the relatively recent realization of this non motor-symptom of PD in routine clinical testing. Anxiety can burden the disease by significantly worsening parkinsonian motor symptoms [4] and negatively impacting quality of life [5,6]. In fact, anxiety is also one of the best predictors of poor quality of life in PD and has been rated more harmful to well-being and more functionally detrimental to PD patients than the motor symptoms of this disease [7,8].

Abbreviations: LA, low anxiety; HA, high anxiety; 6-OHDA, 6-hydroxydopamine; EPM, elevated plus maze; MFB, medial forebrain bundle; OFT, open field test; PD, Parkinson's disease; SNc, substantia nigra compacta.

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While anxiety disorders in PD could be intuitively attributed to the diagnosis of a progressive and chronic neurodegenerative disease, it is still more prevalent in PD than in other chronic medical illnesses such as multiple sclerosis [1] and in the general population [9]. Several preclinical studies have also shown an increase in anxiety-like behavior in PD animal models where the psychological impact of living with a chronic disease is not a factor [10–12]. Additionally, while anxiety can occur at any stage of the disease [13], retrospective studies show that anxiety may serve as an early risk marker for PD since this non-motor symptom manifests during the prodromal stage, many years before the onset of motor symptoms and prior to the diagnosis of PD [10,14–17].

Since little attention has been given to understanding anxiety disorders in PD, there is a lack of awareness and knowledge about this non-motor symptom of PD and information from controlled trials for directing the best treatment option is lacking [18]. Therefore, it is not surprising that anxiety is under-diagnosed and untreated, with one study reporting 53% of PD patients remain un-medicated [19]. This has posed a critical barrier and challenge in developing effective treatment options for anxiety in PD and only a few studies have examined anxiety in preclinical rodent models of PD [10–12,17,20–25]. Even fewer have tested whether typical anxiolytic medications, such as selective serotonin reuptake inhibitors, can diminish anxiety-like behavior with results showing little to no improvement [26,27].

To date, the first-line treatment strategy for anxiety disorders specific to PD has not been established. Instead, current management strategies originate from personal observational studies and published guidelines based on the general public. Furthermore, there is no data to support any drug treatment for PD anxiety and the rationale is based on side effect profiles rather than efficacy [18,28]. In this study, we sought to evaluate anxiety-like behavior in a 6-hydroxydopamine (6-OHDA) late-stage rat model of PD since it is uncommon for anxiety disorders to be diagnosed during the prodromal stage of the disease [13], and assess whether acute administration of diazepam would provide anxiolytic efficacy for two important reasons. First, diazepam is a benzodiazepine commonly prescribed as an anxiolytic for anxiety disorders in the general elderly population [29], but little data exists in clinical and preclinical literature regarding its efficacy in the acute treatment of PD anxiety. Second, the acute nature of diazepam efficacy provides a pragmatic approach in preclinical studies to reveal differences in anxiolytic responsiveness. Ultimately, findings from this study could reveal diazepam's utility and/or limitation as a potential acute treatment option to alleviate anxiety in PD.

2. Materials and methods

2.1. Rat model of PD

Animal use was conducted in accordance with the Albany Medical College Institutional Animal Care and Use Committee consistent with the National Institutes of Health guide for the care and use of Laboratory animals and all efforts were taken to minimize animal suffering and the number of animals employed in this study. Hemiparkinsonian rats were created by lesioning the right medial forebrain bundle as described previously [30]. Briefly, adult male Sprague Dawley rats (n = 164, 200–250 g, Taconic Farms, Rensselaer, NY) were anesthetized with 2% isofluorane (Harvard Apparatus inhalant system, Holliston, MA) and injected intraperitoneally with desipramine (25 mg/kg) and pargyline (50 mg/kg) 20 min prior to the craniotomy. Eye lubricant (Major Pharmaceuticals, Livonia, MI) was applied to prevent dehydration, 2% lidocaine gel (Akorn Pharmaceutics, Lake Forest, IL) was applied to the ear bars and 0.5% bupivacaine (Hospira, Lake Forest, IL) was injected subcutaneously under the shaved scalp to minimize discomfort. A burr hole was drilled above the MFB in the right hemisphere so that 4.5 μl of 6-hydroxydopamine hydrobromide (6-OHDA, 3 μg/μl, made in 0.1% ascorbic acid, n = 101) or saline (0.9% NaCl, n = 63) could be injected into the right MFB (from bregma: 4.4 mm posterior, 1.2 mm lateral, 7.5 mm ventral from dura [31]) at 0.5 μl/min with a 10 μl Hamilton syringe (Hamilton Company, Reno, NV). Ten minutes after injection the incision was closed with surgical staples and covered with triple antibiotic ointment (Actavis Mid Atlantic LLC, Lincolnton, NC). Post-operative care included penicillin (80 μg/kg) in 1 ml of saline injected subcutaneous once immediately after surgery and burprenorphine (0.12 g/kg) injected subcutaneous every 12 h for 72 h.

2.2. Behavioral assays

For all behavioral assays, animals were transported to a separate red-light room between the hours of 7–11 a.m. They were left to acclimate in the room for 1 h prior to any testing except for locomotor activity, which was assessed under white light.

2.2.1. Locomotor activity

Two weeks after 6-OHDA or saline injections, we assessed motor function to confirm that the lesioning surgery elicited forelimb akinesia in our preclinical rat model of PD and not in sham rats. The limb-use asymmetry test (LAT) involved placing a rat in an upright, clear, cylindrical tube for 5 min and counting the number of right or left forepaw touches on the cylinder wall. Marked degeneration of dopaminergic neurons in the right striatum coincides with a touch bias of ≥80% with the right, unimpaired paw (# right touches/# total touches × 100) [32]. This touch bias was used to classify rats as hemiparkinsonian (n = 66). Six other rats had <80% right forepaw touch bias, but were included after post hoc striatal or substantia nigra compacta (SNc) TH immuno-reactivity showed >80% and >50% depletion, respectively (see Section 2.3). Therefore, 72 out 101 total rats that received 6-OHDA injection (71%) were deemed hemiparkinsonian. Other rats were excluded from the study.

2.2.2. Elevated plus maze

Two days after the LAT, rats were tested in the elevated plus maze (EPM), which is a common approach for assessing rodent anxiety-like behavior [33–35]. The EPM is a plus-shaped wood platform raised 52 cm off the ground with two opposing open and two opposing closed arms which are surrounded by opaque walls on three sides made of black plexiglass (50 × 10 × 14: L × W × H in cm). Rats were placed on the central platform facing an open arm and allowed to explore the maze for 5 min. An arm entry occurred when all four paws crossed the threshold from the center into one of the arms. We noted the animal's time spent in the open arm and number of open and closed arm entries. After 4–7 days post-baseline EPM testing, rats were retested in the EPM 15 min after a subcutaneous injection of only diazepam (1.5 mg/kg) or only saline at a volume of 1 ml [36,37]. Previous studies showed that repeated testing of rodents in the EPM can decrease open arm entries or diminish benzodiazepine efficacy [38–40]. However, while our rats were tested twice, we did not observe an effect from the previous exposure since baseline open arm entries (1.3 ± 0.28, n = 42) did not differ from open arm entries in a subsequent test with a saline injection (1.7 ± 0.36, n = 42) (p = 0.295, paired t-test, data not shown) and diazepam efficacy was not diminished (Fig. 3). Presumably, this is due to our limited rat handling prior to EPM testing [41]. After removal of each rat, the maze was sprayed with MB-10 (sodium chlorite and sodium dichloroisocyanurate dehydrate, Quip laboratories, Inc., Wilmington, DE) and wiped dry. All