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Pharmacologically distinct pramipexole-mediated akinesia vs. risk-taking in a rat model of Parkinson's disease

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Abstract

Pramipexole and ropinirole are dopamine agonists that are efficacious in treating motor disturbances of neuropathologies, e.g., Parkinson's disease and restless legs syndrome. A significant portion of treated patients develop impulsive/compulsive behaviors. Current treatment is dose reduction or switching to an alternative dopamine replacement, both of which can undermine the motor benefits. Needed is a preclinical model that can assist in identifying adjunct treatments to dopamine agonist therapy that reduce impulsive/compulsive behaviors without interfering with motor benefits of the dopamine agonist. Towards that objective, the current study implemented a rat model of Parkinson's disease to behaviorally profile chronically administered pramipexole. This was accomplished with male Sprague-Dawley rats wherein (i) 6-hydroxydopamine-induced lesions of the dorsolateral striatum produced Parkinson's disease-like akinesia, measured in the forelimbs, (ii) intracranial self-stimulation-mediated probability discounting indicated impulsivity/risk-taking, and (iii) two doses of pramipexole were continuously administered for 14–28 days *via* osmotic minipumps to mirror the chronic, stable

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exposure achieved with extended release formulations. The atypical antidepressant, mirtazapine, is known to reduce behaviors associated with drug addiction in rats, thus, we demonstrated model utility here by determining the effects of mirtazapine, on pramipexole-induced motor improvements *versus* probability discounting. We observed that forelimb akinesia subsequent to striatal lesions was attenuated by both pramipexole doses tested (0.3 and 1.2mg/kg/day) within 4hr of pump implant dispensing 0.3mg/kg/day and 1h by 1.2mg/kg/day. By contrast, 12–14 days of infusion with 0.3mg/kg/day did not alter discounting, but increases were obtained with 1.2mg/kg/day pramipexole, with 67% of 1.2mg/kg/day-treated rats meeting categorical criteria for ‘high risk-taking’. Insertion of a second minipump delivering mirtazapine did not alter motor function during 14 days of co-administration with pramipexole, but was sufficient to attenuate risk-taking. These outcomes revealed distinct probability discounting and anti-akinesia profiles for pramipexole, indicating that pharmacotherapy, (e.g., mirtazapine treatments), can be developed that reduce risk-taking while leaving motor benefits intact.

Keywords

impulsivity; mirtazapine; Parkinson’s disease; pramipexole; probability discounting

INTRODUCTION

Agonists of the dopamine D2 receptor family (D2R) (e.g., pramipexole (PPX), ropinirole) are efficacious in treating motor deficits of Parkinson’s disease (PD) and restless legs syndrome. However, dopamine agonist therapy is associated with the development of impulsive/compulsive disorders (ICDs) in a significant portion of treated patients [1–3]. These disorders can be emotionally and financially devastating for the afflicted individuals and their families. Therapeutic regimens that treat motor deficits but do not induce ICDs are needed; however, relevant alternatives include adjunct treatments that can mitigate established ICDs, without impacting the capacity of a dopamine agonist to improve PD. For this to be feasible, the brain substrates engaged by D2R agonists that mediate motor benefits must differ from those that mediate ICDs. To functionally dissect these brain substrates, we developed a sophisticated paradigm that allows for concurrent testing of PD-like motor pathology (akinesia) and impulsivity related to ICDs observed in vulnerable dopamine agonist-treated PD patients.

Impulsiveness includes a greater tendency for risk-taking. This tendency can be quantified using probability discounting procedures; a cross-species assessment in which the subject chooses between a small reinforcer (SR) delivered at a high probability, and a large reinforcer (LR) delivered at a low probability. Typically, the subjective value of the LR is discounted when its probability is low, and individuals then select the smaller, yet more certain reinforcer. In western cultures, problem/pathological gambling, hypersexuality, and impulsive/compulsive shopping and eating, are the most frequently reported ICDs in PD [1–3]. Those who suffer from these disorders are less sensitive to low probabilities than healthy individuals, and they more frequently select the LR even when the odds to obtain the reward are very low [4–8].

Discounting tasks also have been implemented in rats, using food [9] or intracranial self-stimulation (ICSS) [10–13] as positive reinforcers. We demonstrated that ICSS protocols allow for repeated testing without changing feeding status, and we have implemented this procedure in a rat model of PD (i.e., rats with 6-hydroxydopamine (6OHDA)-induced lesions of the dorsolateral striatum (DLS)) [8,10,11]. We also determined that repeated, intermittent intraperitoneal treatments of relatively high doses PPX increases ICSS-mediated probability discounting [10]. Outbred rats exhibit differential sensitivities to moderate doses of indirect acting dopamine agonists, e.g., cocaine and amphetamine, and the treated rats are commonly categorized as high or low responders [14]. Accordingly, we postulated here that moderate doses of PPX might enhance risk-taking only in a subset of (more sensitive) rats. Such a scenario would parallel the human condition wherein only some PPX-treated PD patients develop ICDs [2,3].

The present study was designed to determine if, in an outbred strain of rats, a dose of PPX that mitigated 6OHDA-induced forelimb akinesia in all rats would concurrently increase probability discounting only in some. As motor benefits of agonist therapy in PD are typically realized long before diagnosis of ICDs [15], we also determined the onset of PPX-induced effects on akinesia and probability discounting. Extended release formulations of PPX are frequently used for human patients to provide relatively stable exposure to the drug during chronic therapy [16]. To emulate this therapeutic approach in rats, we evaluated continuously delivered PPX using subcutaneously (sc) implanted osmotic minipumps.

Currently, there are no FDA-approved pharmacotherapies for ICDs with neurological comorbidities. Accordingly, another objective of the current study was to validate the utility of our PD/risk-taking model to help identify putative adjunct therapies that maintain dopamine agonist-mediated motor improvement but reduce ICD-like behaviors. Brain and behavioral substrates of ICDs are similar to those engaged during substance use disorders and drug addiction [17]. Thus, we postulated that therapies which hold promise for addiction may provide relief from ICDs. Mirtazapine, an atypical antidepressant, reduces behaviors associated with substance use disorders in rats [18–22] and humans [23,24]. Moreover, mirtazapine has been used to treat anxiety, depression, and psychosis in PD patients, and is well-tolerated by PD patients on dopamine agonist therapy with little to no effect on the motor benefits afforded by the agonist [25–30]. This latter feature likely reflects the lack of mirtazapine binding to dopaminergic receptors [31,32]. Based on this backdrop, we tested the utility of mirtazapine to diminish PPX-induced risk-taking in rats.

The hypotheses for the study were that (i) chronic, continuous delivery of low to moderate doses of PPX will reverse forelimb akinesia induced by 6OHDA lesions of the DLS, (ii) some treated rats will exhibit increased risk-taking in the probability discounting task, and (iii) mirtazapine will attenuate risky behavior, but not the motor benefits of PPX.

METHODS

Animals and Surgical Procedures

Male Sprague-Dawley (outbred) rats (250–300g; Harlan Laboratories, Indianapolis, IN) were pair-housed under environmentally controlled conditions under a 12h light/dark cycle

(lights on at 07:00); food and water were available *ad libitum*. Experimental procedures were approved by the IACUC at Rush University Medical Center and in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals”. Experimental timelines are illustrated in Fig. 1.

A commonly used rat model of PD that captures several critical features of the disease involves infusion of 6OHDA into the dorsolateral striatum (DLS) [33]. The DLS is the homolog of the human putamen, the area of the brain most severely affected during early stages of PD. As Dopamine agonists are frequently used to treat early stage PD, we employed this lesion profile for the current study. Following our published protocols [10,13], desipramine (Sigma-Aldrich), a norepinephrine reuptake inhibitor, was administered (25mg/kg subcutaneously (sc)) to enhance dopaminergic selectivity of 6OHDA. Rats were anesthetized with isoflurane and secured in a stereotaxic frame. 6OHDA (7 μ g) was bilaterally infused (0.2 μ L/min for 10min) into the DLS (from Bregma: +1.0mm AP, $\pm$ 3.4mm ML; -4.7mm DV from skull). We previously verified that this protocol depletes tyrosine hydroxylase (TH) consistently confined to the DLS [10,13,34], and retrogradely reduces the number of TH⁺ neurons within the lateral aspect of the substantia nigra *pars compacta* by 50% [13]. This 6OHDA-induced lesion profile results in a predictable, severe forelimb akinesia in all rats which persists for at least 3 months post-injection [13]. After the 6OHDA injection, a bipolar stimulating electrode (MS303/3-B/SPC; Plastics One, Roanoke, VA) was lowered to the lateral hypothalamus (LH) for ICSS (from Bregma: -2.6mm AP; -1.8mm ML; -8.4mm DV from skull), and the electrode plug was held in place with dental acrylic affixed to stainless steel screws secured in the skull. At least one week recovery time was allowed before initiating operant task training. We previously verified that ICSS does not occur if electrode tips are located outside the LH, and that tip location within the LH does not correlate with ICSS-mediated outcomes [9].

Test Drug Treatments

Therapeutically effective PPX doses (i.e., 1.75–6.5mg/70kg human/day) [35] were used for standard conversion methods to determine dosing equivalence for rats [36]. This, and our prior discounting study with 2mg/kg/day PPX (total dose per twice daily intraperitoneal injections) [10] guided the choice of testing chronically infused 0.3 and 1.2mg/kg/day (+)PPX HCl (NIMH Drug Supply Program, Rockville, MD) for the current study. PPX was dissolved in sterile saline and delivered sc *via* osmotic minipumps (model #2002 or #2ML4; Alzet, Cupertino, CA).

To determine the effects of mirtazapine on PPX-induced behaviors, rats treated with 1.2mg/kg/day PPX received a second minipump that delivered mirtazapine (NIMH) or its vehicle solution. Mirtazapine was dissolved in 1N HCl, diluted with sterile water, brought to 5.5–6.0pH with 1N NaOH and delivered as 5mg/kg/day. We previously determined in rats that this dose of mirtazapine does not alter locomotor function [18] but is sufficient to reduce several measures of drug reward-motivated behavior [18–22].

Minipumps were inserted sc in the mid-scapular region under isoflurane anesthesia per the manufacturer’s directions. For PPX, this occurred after stable baseline (BL) discounting

(defined below) was established (Fig. 1A&B). For mirtazapine (or its vehicle solution), this occurred after the discounting session on day 14 of PPX treatment (Fig. 1B).

Behavioral Protocols

Akinesia occurs in early stages of PD, and this motor deficit is modeled in rats using a forelimb step task [33,37]. Thus, we utilized this task as motor index of the 6OHDA-induced lesion and PPX treatment efficacy, per our previously published procedures [10,13]. In brief, the experimenter gently restrained the rat's rear legs and one forelimb, and 'dragged' the rat on the unrestrained forepaw 0.9m for 5s in the abduction direction. The number of steps was counted for three trials and the average determined. As previously shown [10], there was no difference in the number of adjusted steps between left and right forepaw; data from the left forepaw are reported. The test was conducted before (termed pre-lesion), and approximately two weeks following 6OHDA injection (post-lesion), as well as on various days during the chronic treatments (Fig. 1A&B). Randomly selected rats were tested 0.5–4h immediately following PPX minipump implant to determine the onset of the drug effect.

Operant paradigms were conducted using Med-Associates (St. Albans, VT) chambers. Parameters for the ICSS-mediated probability discounting task were previously established following extensive assessments of individual rat preferences [10–12]. In overview, ~7days after intra-DLS 6OHDA injections, rats were trained to press a lever for brain stimulation (200 μ s biphasic square wave pulses delivered at 100Hz for 500ms), starting at 100 μ A. These are typical values that successfully support discounting operant tasks [9], but lever pressing was optimized for each rat by adjusting the current in 20 μ A increments. The optimal current intensity was used in a fixed ratio-one schedule of reinforcement to establish self-initiation and maintain high rates of stable lever pressing (>8 lever presses/min). Rats then were required to discriminate between levers associated with either a SR or a LR [13]. The sessions consisted of two blocks of 20 forced and 20 free-choice trials. During forced trials, one lever was extended at a time, with lever assignments for LR vs. SR alternating between trials; during free-choice trials, both levers were extended and rats were allowed to select between them. The SR started at 70Hz, the LR started at 120Hz; these values were adjusted in 10Hz increments as needed (while maintaining at least 50Hz difference between the SR and LR) to achieve >80% response preference for the LR over three consecutive sessions of free-choice trials. The rats then were advanced to the probability discounting task wherein they selected between SR-associated levers delivered at a probability of 1.0 and LR-associated levers delivered at probabilities of 0.8, 0.5, 0.2, and 0.1, presented in descending order. Each session consisted of 5 blocks with 20 trials per block. The first block consisted of forced trials as in the discrimination task, and each subsequent block consisted of free-choice trials at each differing probability. Rats were randomly assigned to receive the LR on either the left or right levers for all sessions. The primary outcome was the number of selections made for the LR divided by total selections $\times$ 100 for each probability. Stability, defined as <50% response omissions and <15% variability for three consecutive sessions, was established for each probability. Plotting the %LR vs. probability yielded a discounting curve wherein %LR responding at the 0.2 and 0.1 probabilities fell below the indifference point. Accordingly, rats were categorized as high risk-takers if they demonstrated a >15% increase in LR selection at the 0.2 and/or 0.1 probabilities using the average of PPX

treatment days 12–14, compared to BL. Rats with <15% change were categorized as low risk-takers. Likewise, a mirtazapine effect was categorically based on >15% change following 14 days of co-infusion with PPX, compared to PPX alone in the high risk-takers.

Statistical Analyses

One-way or multi-way repeated measures (rm) ANOVAs were used to analyze data and Newman-Keuls tests were used for *post hoc* comparisons. Significant differences are illustrated for the *a priori* identified individual comparisons (i.e., planned contrasts). A Fisher's exact test was used to compare mirtazapine or vehicle treatment for high risk-takers vs. low risk-takers. Data were analyzed using Graphpad Prism 5.0 (Graphpad Software, La Jolla, CA) or GB-Stat v10 (Dynamic Microsystems, Inc, Silver Spring, MD) software, and are presented as mean±SEM or mean+SEM.

RESULTS

We [10,13] and others [33] show that deficits in the forelimb step task (i.e., akinesia) reflect substantial reductions of DLS TH (lesion illustrated in Fig. 1C). Likewise, here, intra-DLS 6OHDA produced akinesia, (i.e., an ~80% reduction in stepping) in all rats, and this was attenuated with PPX infusion at 0.3 and 1.2mg/kg/day (e.g., Fig. 2A). Evaluating effect onset; there was a main effect of time ($F_{6,78}=144.86$, $p<0.0001$), and a dose×time interaction ($F_{6,78}=4.22$, $p=0.001$), but no dose effect. While both doses of PPX relieved 6OHDA-induced forelimb akinesia (i.e., significant differences were obtained between 6OHDA-induced stepping deficits and stepping during PPX-treatment), the 1.2mg/kg/day dose was more effective at 2h and 3h after implanting the PPX minipump compared to 0.3mg/kg/day. These outcomes illustrated the validity of the PD model, and verified that the minipumps delivered PPX so that physiologically effective doses were reached sooner with the higher infused concentration. It is noteworthy that we did not observe dyskinesia during chronic treatment with PPX up to 1.2mg/kg/day in any rats tested.

Initial evaluations of probability discounting (n=14 rats) indicated PPX dose-related differences in the selection of the LR at the 0.2 and 0.1 probabilities. Two weeks treatment with 1.2mg/kg/day resulted in significant increases in discounting compared to baseline (i.e., increased selection of the LR, indicating more risk-taking), whereas 0.3mg/kg/day did not affect selection of the LR (Fig. 2B): At the 0.2 probability, rmANOVA revealed an effect of dose ($F_{1,12}=30.58$, $p=0.0001$) and an effect of time ($F_{1,12}=6.01$, $p=0.031$), but no interaction. At the 0.1 probability, there was a significant effect of dose ($F_{1,12}=6.14$, $p=0.029$), with a trend toward a significant effect of time ($F_{1,12}=4.2$, $p=0.06$), but no interaction. For 1.2mg/kg/day PPX, the selection of the LR was increased as compared to BL or 0.3mg/kg/day PPX. As a result, we selected the 1.2mg/kg/day PPX dose for the remaining experiments.

Not all PPX-treated PD patients develop ICDs. We were interested in determining if this phenomenon was reflected in PPX-treated, 6OHDA-lesioned rats during the ICSS-mediated probability discounting tasks. Using all rats tested with 1.2mg/kg/day PPX and discounting data collected on days 12–14 of treatment (see Fig. 1A&B), we grouped the rats into high risk-takers or low risk-takers (see Methods); 67% met criteria for high risk-taking (22/33

tested). Discounting curves for high and low risk-takers are illustrated in Fig. 3A. It is noteworthy that the difference in magnitude between high and low risk-takers is similar to the reported difference between high and low responding Sprague-Dawley rats in a delayed discounting task [38]. Subjecting the probability discounting data to a 3-way rmANOVA revealed significant differences between high- and low-risk groups ($F_{1,31}=17.72$, $p=0.0002$), treatment (BL vs. PPX; $F_{1,31}=33.40$, $p<0.0001$) and probability of LR delivery ($F_{3,93}=342.27$, $p<0.0001$), as well as a risk group×treatment interaction ($F_{1,31}=38.44$, $p<0.0001$), a treatment×probability interaction ($F_{3,93}=3.93$, $p=0.011$), and a risk group×treatment×probability interaction ($F_{3,93}=3.93$, $p=0.01$). There was no difference in BL discounting between risk groups; however, following chronic PPX treatment, high risk-takers showed increased discounting (i.e., increased selection of the LR) at the 0.5 ($n=12/22$), 0.2 ($n=19/22$) and 0.1 ($n=11/22$) probabilities, but not the 0.8 probability ($n=5/22$), compared to BL. To determine if ICSS reward value differed between risk groups, or during PPX exposure, we compared the stimulation frequencies used for the SR and LR between high and low risk-takers, for days 1 and 14 of 1.2mg/kg/day PPX. There were no significant differences between risk group, time, or a risk group×time interaction for either reinforcer (Fig. 3B), indicating that reward perception was not different between groups and was stable for the duration of PPX treatment. We also determined if the capacity for 1.2mg/kg/day PPX to improve 6OHDA-induced akinesia differed between risk groups for days 1 and 14 of PPX infusion (Fig. 3C). There was a significant effect of time ($F_{3,90}=190.29$, $p<0.0001$), but not treatment, nor interaction. *Post hoc* tests indicated that PPX improved akinesia in both low and high risk-taking rats at days 1 and 14, and there was a significant difference between risk groups on day 14. This suggests that high risk-takers may be more sensitive to the motor benefits of chronic PPX. To determine the onset of PPX-induced risk-taking, the first three discounting sessions from high risk-takers were analyzed using a one-way rmANOVA; onset of PPX-induced discounting was determined by comparing to BL while response stability was determined by comparing to PPX day 14 (Fig. 3D). There was a significant effect of PPX on discounting at the 0.2 and 0.1 probabilities ($F_{4,84}=2.77$, $p<0.0001$; $F_{4,84}=4.64$, $p=0.008$, respectively). By day 2 of PPX treatment, there was a significant increase in selection of the LR at both 0.2 and 0.1 probabilities, and the PPX-induced increase was stable for the duration of PPX treatment (i.e., day 3 did not differ from day 14).

We hypothesized that administration of mirtazapine would reduce risk-taking behavior induced by chronic PPX treatment. To test this hypothesis, we categorized the rats' discounting on day 14 of PPX treatment and then implanted high risk-taking rats ($n=14$) with a second minipump, randomly assigned to deliver mirtazapine ($n=7$) or its vehicle ($n=7$) (see Fig. 1B). Discounting behavior was assessed for an additional 14 days. Two rats (one from each group) were excluded due to occlusion of the minipump or high omissions. The effects of mirtazapine or vehicle co-administration were evaluated for PPX days 26–28 (Fig. 1B), and outcomes were compared to discounting on days 12–14 of PPX (only) and BL at the 0.2 or 0.1 probabilities (Fig. 4A). For the 0.2 probability, there was an effect of time ($F_{2,20}=36.80$, $p<0.0001$), but no treatment effect or interaction. PPX increased discounting on days 12–14 compared to BL. At the 0.1 probability, there were no significant main effects; however, there was a trend toward significance for time ($p=0.09$) and for a

treatment×time interaction ($p=0.08$). While not detected by the rmANOVA, *post hoc* tests revealed trends for mirtazapine to reduce PPX-enhanced discounting for both the 0.2 and 0.1 probabilities (indeed, paired *t*-tests were significant ($t_5=2.37$, $p=0.032$; $t_5=2.73$, $p=0.021$, respectively). Using a >15% reduction in PPX-induced discounting as the categorical criterion (see Methods), a one-tailed Fisher's exact test revealed that the number of PPX-responding rats was significantly reduced during mirtazapine co-administration (i.e., 5/6 for mirtazapine vs. 0/6 for vehicle; $p=0.008$; Fig. 4B). In contrast, mirtazapine had no effect on PPX-induced improvements in akinesia (Fig. 4C). Finally, akinesia was reinstated following removal of the pumps (Fig. 4C), substantiating the link between motor improvements and the presence of PPX.

DISCUSSION

This study demonstrates in rats (i) the utility of a minipump chronic administration protocol, (ii) the ability of PPX to reduce forelimb akinesia imposed by 6OHDA-induced lesions of the DLS (iii) vs. promoting risk-taking behavior, and (iv) the utility of a non-food motivated, probability discounting task. Study outcomes support the possibility that dopamine agonists engage different neuronal systems and/or biochemical processes for these two behavioral functions. The efficacy of this novel PD/risk-taking model to distinguish between changes in motor and risky behaviors during sustained exposure to a dopamine agonist reveals a viable platform for assessing the proclivity of new compounds that may benefit PD-like motor pathology to also impart unwanted ICD-like side effects. Finally, the outcomes with mirtazapine provide proof-of-concept for using this model to evaluate potential adjunct therapy in dopamine agonist-treated PD patients that can reduce ongoing risk-taking/ICDs without interfering with the motoric benefits of dopamine agonists.

Several pharmacological distinctions were observed between PPX-induced improvements in akinesia (i.e., forelimb adjusted stepping) and risk-taking (i.e., ICSS-mediated probability discounting). One difference was the *capacity* of PPX to regulate these behaviors. Motor benefits were observed for all rats at both doses tested. The benefits were present within 1h of implanting minipumps delivering 1.2mg/kg/day PPX, and maximal efficacy was reached by 24h. In contrast, increased discounting was only achieved with 1.2mg/kg/day PPX, with a subset (i.e., 67%) of these rats meeting criteria for risk-taking, and maximal efficacy was not obtained until day 2 of PPX treatment. PPX effects on both akinesia and discounting were maintained for the duration of the treatment paradigm, indicating tolerance did not occur during sustained exposure. These results are consistent with the idea that in the PD-like brain state, where striatal regions are dopamine-deafferented, motor improvement afforded by dopamine agonists are acting on upregulated postsynaptic dopamine receptors to rapidly provide more normal function of striatal outflow to downstream motor structures. The limbic brain regions involved in ICDs are largely intact in PD [39] (but see [40,41]) and in rats with 6OHDA-induced lesions of the DLS [10,13], and Dopamine agonist therapy for PD may serve to over activate limbic dopamine receptors. Furthermore, chronic exposure to PPX upregulates D3R [42]. These factors may underlie an enhancement in D2/D3R-function that is associated with enhanced vulnerability for ICDs [43,44]. Thus, with chronic PPX, the imbalance in limbic dopamine transmission likely reaches a critical state, and ICDs become realized in vulnerable individuals.

We demonstrated the utility of this PD/risk-taking model for evaluating combination drug treatment therapy by determining the impact of chronic mirtazapine infusions on PPX-induced motor improvements vs. risk-taking behavior. As summarized in Fig. 5, chronic mirtazapine infusion did not alter the improvements in akinesia provided by PPX; however, PPX-enhanced risk-taking was attenuated (albeit, modestly). This trend is in keeping with the ability of mirtazapine to reduce motivated behaviors sustained by abused drugs in rats and humans [18–22,24,45] and ICSS-mediated decision-making in rats [46]. Outcomes also indicate that the systems engaged by mirtazapine may be more relevant to ICD than motor function. Mirtazapine exhibits high affinity for α_2 adrenergic receptors and several serotonergic receptors, including 5-HT_{2A/2C}. Evidence is accumulating for the involvement of these receptors in ICDs [47,48], including ICDs in PD [49], but the precise role remains controversial. Norepinephrine and serotonin regulate dopamine transmission, and it may be that normal limbic dopamine transmission balance involves multiple serotonergic and adrenergic receptors, and the multi-site action of mirtazapine helps reinstate this balance in the PPX-treated, PD-like brain state. Several adrenergic and serotonergic agonists are garnering attention for reducing impulsivity in laboratory rats and humans [50–52], additional experimentation with the current model using receptor-selective compounds will be particularly informative. Nonetheless, given the clinical reports showing the efficacy and tolerability of mirtazapine for treating psychiatric disorders in PD patients [25–30], our results indicate that further investigations into the utility of mirtazapine for treating PPX-induced ICDs are warranted.

Several nuances of the study outcomes are worth highlighting. First, various assessments demonstrated the utility of the minipump delivery system for PPX, including dose-related onset and stability behavior effects, and a return to pre-PPX levels of 6OHDA-induced akinesia one day after pump removal. Second, like the human counterpart [2,3], only a portion of the rats who were treated with, and exhibited motor benefits from PPX, subsequently developed a high-risk profile in the probability discounting task. The proportions reported for humans are 13–35% [2,3]; considerably lower than the 67% observed in our rats. In humans, ICD profiles reflect pre-existing vulnerabilities, epigenetics, influences of context and environment, and/or comorbidities, as well as the capacity to diagnose the particular impulsive/compulsive behavior (which has been historically under reported). These features are not recapitulated in Sprague-Dawley rats, so it is not unexpected that the distribution would differ between rats and humans. Moreover, a primary objective for the current study was to determine if combination therapies (e.g., PPX + mirtazapine) could be tested for reductions in PPX-induced risk-taking, and a relatively large portion of PPX-sensitive rats was useful in efficiently accomplishing this objective. Third, it was interesting that high risk-takers exhibited slightly greater PPX-induced motor benefits than low risk-takers. DLS lesion-imposed retrograde degeneration of dopaminergic neurons is time-dependent, and we verified that the number of TH⁺ neurons in the dorsolateral *pars compacta* is reduced to 50% by 90 days post-lesion [13]. Our protocols result in remarkably consistent DLS TH reductions and the associated akinesia, and probability and delayed discounting are extremely stable for at least 90 days post-lesion [8,11]. Thus, behavioral outcomes of our prior and current studies do not reflect the extent of dopaminergic cell loss, and the current observations suggest a general higher sensitivity to DAs by risk-takers that

involves other factors. Finally, it is noteworthy that a two week treatment with 5mg/kg/day mirtazapine did not completely reverse PPX-mediated risk-taking behavior, and it may be that a longer duration or higher doses of mirtazapine treatment is necessary to achieve full reversal.

CONCLUSION

In conclusion, we confirmed the differing pharmacological profiles for akinesia and risk-taking following PPX treatment in DLS-lesioned rats, a rat model of PD. PPX-induced risk-taking was blunted by the concomitant administration of mirtazapine while the motor benefits remained intact. These results illustrate the utility of this rat model of PPX-induced motor benefits and risk-taking/ICDs in PD patients. The results also indicate the merit of additional research into the potential utility of mirtazapine and other α_2 and/or 5-HT_{2A/2C} ligands as adjunct therapies for dopamine agonist-treated patients that developed ICDs.

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HIGHLIGHTS

- A novel model of impulsivity associated with Parkinson's disease is described.
- Dose-related effects of continuously released pramipexole in this model are revealed.
- Pramipexole effects on motor dysfunction were distinct from effects on impulsivity.
- Mirtazapine altered impulsivity, but not motor benefits, of pramipexole.
- The model may help identify adjunct therapy to reduce unwanted side effects of pramipexole.

Figure 1.

Experimental timeline and verification of striatal lesion. **(A)** Illustration of timeline for PPX-induced risk-taking. The DLS was lesioned with 6OHDA and implanted with an ICSS electrode targeted at the medial forebrain bundle. Rats then were trained for ICSS followed by probability discounting. Once a stable baseline was obtained, rats received a subcutaneous osmotic minipump that released PPX at a rate of 0.3mg/kg/day or 1.2mg/kg/day. PPX was infused for 14 days, during which probability discounting was assessed each day. Forelimb akinesia was evaluated before lesion, after lesion, and at various time points during PPX administration. **(B)** Illustration of experimental timeline for the effects of mirtazapine on forelimb akinesia and PPX-induced risk-taking. The protocol was the same as in (A) with the exception that PPX (1.2mg/kg/day) was infused for a total of 28 days. On day 14 of PPX treatment, a second osmotic minipump was implanted that released mirtazapine at a rate of 5mg/kg/day for 14 days. Probability discounting was assessed each day of chronic drug treatment; forelimb akinesia was evaluated before lesion, after lesion, and at various time points during chronic drug administration. **(C)** Representative coronal photomicrographs of tyrosine hydroxylase immunoreactivity. Illustrating typical bilateral treatments, shown are unilateral sections within the DLS of a sham treatment (left) and a 6OHDA-induced lesion (right) 60 days after injection.

Figure 2.

Pramipexole administration improved forelimb akinesia and increased risk-taking in Parkinson's disease-like rats. **(A)** The onset of PPX-induced improvements in forelimb akinesia was dose-dependent. With 1.2mg/kg PPX, akinesia was significantly improved compared to lesion alone (6OHDA) within 1hr of pump implant. In contrast, 0.3mg/kg/day PPX did not improve akinesia until 3hr after pump implant. (†, different from respective 6OHDA; *, different between PPX doses). **(B)** Chronic administration of PPX increased risk-taking at the 0.2 and 0.1 probabilities in rats treated with 1.2mg/kg/day, but not 0.3mg/kg/day. Baseline (BL) was obtained with the lesion alone. (†, BL vs. 1.2mg/kg/day. *, different between doses).

Figure 3.

Chronic pramipexole administration (1.2mg/kg/day) increased risk-taking in a subset of Parkinson's disease-like rats. (A) Rats were categorized as high risk or low risk (see Methods). In high risk rats, 14 days of PPX treatment increased discounting at the 0.5, 0.2 and 0.1 probabilities. In contrast, PPX had no effect in low risk rats. Baseline (BL) was obtained with the lesion alone. (**, high risk BL vs. PPX). (B) ICSS current frequency for the small and large reinforcers was stable for the 14 days of PPX administration and did not differ between high risk and low risk rats. (C) Chronic PPX treatment improved forelimb akinesia in both high risk and low risk rats. (††, different from respective 6OHDA lesion alone. **, different between high and low risk groups). (D) In high risk rats, onset of PPX-increased discounting occurred at low probabilities within 2 days of treatment; this effect was maintained through day 14 of PPX administration. Baseline (BL) was 6OHDA lesion alone. (†, different from respective BL. #, different from Day 1).

Figure 4.

Effect of mirtazapine on pramipexole-induced risk-taking and akinesia. **(A)** At the 0.2 and 0.1 probabilities, there was a trend for MIRT to decrease selection of the large reinforcer. **(B)** MIRT reduced the number of rats that met criteria for PPX-induced risk-taking. YES, met criteria. NO, failed to meet criteria. **(C)** PPX-induced anti-akinesia was not affected by MIRT co-administration.

Figure 5.

Distinct effects of pramipexole and mirtazapine on forelimb akinesia (left axis) and risk-taking (right axis). Number of PPX treatment days are illustrated on the horizontal axis. Baseline (BL) refers to 6OHDA-induced forelimb akinesia, or three consecutive discounting sessions prior to PPX treatment. The PPX-induced improvement in forelimb akinesia was maintained following MIRT while PPX-induced risk-taking was reduced.