EFFECTS OF ACUTE PRAMIPEXOLE ON PREFERENCE FOR GAMBLING-LIKE SCHEDULES IN MALE WISTAR RATS

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ABSTRACT

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In recent years, pramipexole and other direct-acting dopamine agonist medications have been implicated in the development of impulsive behaviors such as pathological gambling in patients with Parkinson's disease. Despite the significance of these clinical findings, experimental evaluations of pramipexole's effects on gambling are lacking. To this end, the present study used an animal model approximating some aspects of human gambling to examine within-subject effects of acute pramipexole on rats' preferences for gambling-like sources of reinforcement. Pramipexole modestly but significantly increased preferences for gambling-like reinforcement when compared to saline. Pramipexole also increased response latencies, but did not affect probabilities of response perseveration. The findings of the present study are consistent with clinical reports linking pramipexole to gambling. Results are discussed in the context of neurobehavioral evidence suggesting a critical role for dopamine in reward- and punishment-related learning processes.

Keywords: pramipexole, pathological gambling, dopamine agonist, impulsive behavior, Parkinson's disease, rat, lever press

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Pramipexole is a dopamine (DA) D_2/D_3 receptor agonist commonly prescribed as part of dopamine replacement therapy for Parkinson's disease (PD). Pramipexole has high selective affinity for the D_3 receptor subtype (Bennet & Piercey, 1999) which is predominantly expressed in the limbic areas of the brain (Sokoloff et al., 1990). Limbic areas are thought to mediate aspects of addictions to drugs and gambling (Lader, 2008). Several clinical reports indicate that when some patients with PD are treated with D_2/D_3 agonists, like pramipexole, they develop impulse control disorders (ICDs) such as pathological gambling (Dodd et al., 2005; Driver-Dunckley, Samanta, & Stacy, 2003; Grosset et al., 2006; Molina et al., 2000), compulsive shopping (Giladi et al., 2007), hypersexuality (Giovannoni et al., 2000; Klos et al., 2005; McKeon et al., 2007; Munhoz, Fabiani, Becker, & Teive, 2009), and compulsive eating (Nirenberg & Waters, 2006). The causal role of these agonist medications in the development of impulsive behaviors is suggested by the absence (or socially acceptable frequency) of ICDs prior to drug therapy and the subsequent resolution of the problematic behavior once drug use is discontinued (e.g., Mamikonyan et al., 2008).

Of the aforementioned ICDs, incidence of pathologic gambling in PD patients prescribed D_2/D_3 agonists has been a subject of particular interest (e.g., Imamura et al., 2008; Ondo & Lai, 2008). For example, the Food and Drug Administration's 2005 Adverse Event Reporting System Database listed 67 pramipexole-associated gambling incidents; 58% of the total reported cases of side effects of this drug (Szarfman, Doraiswamy, Tonning, & Levine, 2006). Prevalence estimates of

pathological gambling among PD patients treated with D_2/D_3 agonists range from 0.5-7.2%, higher than a recent U.S. population estimate of 0.4% (Petry, Stinson, & Grant, 2005). Even so, some researchers have speculated that the effects of D_2/D_3 agonists on PD patients' gambling have been underestimated because of inadequate screening procedures, reluctance to admit a problem, lack of context to express gambling behavior, or the exclusion of patients whose problem gambling does not meet pathologic levels (e.g., Potenza, Voon, & Weintraub, 2007).

To our knowledge, only two laboratory experiments have examined the relation between pramipexole and gambling-related activities. Riba, Krämer, Heldmann, Richter, and Münte (2008) presented healthy human participants with a task in which choosing one option resulted in an equally probable (i.e., 50%) win or loss of 25 cents or a "boost" win (i.e., 50 cents) that occurred unpredictably on half of the win trials. Choosing the other option had similar outcomes, but with 5 cents as the amount won or lost (10 cents for a "boost" win). In a comparison of the within-subject effects of 0.5 mg pramipexole vs. placebo in this lottery-style task, Riba and colleagues reported that pramipexole significantly increased persistence for the 25 cent option immediately following a "boost" win at this alternative, despite the fact that wins and losses were equally likely throughout the session (no other comparisons were significant). Pre-session pramipexole also produced hypoactivation of reward-related brain regions (e.g., ventral striatum, cingulate gyrus) which Riba et al. speculated may be related to the blunted responsiveness to risk.

The other experimental examination of the effects of pramipexole on behavior related to addictions was conducted by Hamidovic, Kang, and de Wit (2008) who reported no significant effects of 0.25 or 0.5 mg pramipexole on human impulsivity in delay and probability discounting tasks. In these tasks, participants were asked to choose between a smaller monetary amount available immediately (or with 100% certainty) and a larger amount whose receipt was delayed in time (or less than 100% certainty). Depending on a participant's choices, the smaller monetary amount was adjusted until a point of subjective indifference between the small and large amounts was obtained. The task was then repeated across a range of delay and probability values for the larger amount in order to generate a curve whose steepness is a common metric of impulsivity. Although no significant differences in discounting were found between placebo and drug sessions, their sample of 8 participants demonstrated a dose-dependent trend toward more impulsive choice in the delay discounting task. Consistent with this trend, an experiment recently conducted in our laboratory reveals a significant increase in impulsive choice in rats given pre-session injections of pramipexole at doses of 0.1, 0.18, & 0.3 mg/kg (Madden, Johnson, Brewer, Pinkston, Fowler, & Woods, in preparation). It should be noted, however, that this effect was not reproduced in two subsequent experiments using a different procedure (that developed by Evenden & Ryan, 1996).

These laboratory findings, when combined with the clinical-reports literature on gambling in PD patients, suggest D_2/D_3 agonist medications (like pramipexole) may increase ICDs such as pathological gambling. Additional experimental data is

needed to thoroughly address this hypothesis. Because human research necessarily involves uncontrollable behavioral histories and ethical issues (e.g., participants cannot gamble with their own money), nonhuman assessments of pramipexole's effects on impulsivity may provide insights into this phenomenon.

The present study was designed to explore the effects of pramipexole on gambling-like behavior in rats. We selected a nonhuman laboratory preparation to control subjects' learning history and to make it possible to schedule important reinforcing consequences in a manner similar to the way in which wins occur in human gambling (e.g., Kendall, 1987; 1989). For these purposes, a choice procedure was used to determine if pramipexole increases preference for "gambling" over a predictable source of reinforcement.

Rats completed 4-hour sessions in which they earned their entire ration of food for the day by responding on levers in an operant chamber (i.e., a closed economy). Within each session, rats had a fixed budget (i.e., a finite number of lever-press responses) to "spend" on food each day. Once the rat's budget had been exhausted, the session ended and food was unavailable until the next day's session. Because human pathological gambling frequently results in a net loss of income, conditions were arranged such that food earned from the "gambling" alternative was more expensive than that obtained on a separate lever where food was arranged more predictably. Thus, given the "budgetary limitations" in this preparation, choosing the gambling-like alternative negatively affected food intake (i.e., income) and arranged for the long-term losses commonly associated with human pathological gambling.

Method

Subjects

Seven experimentally naïve male Wistar rats obtained from Charles River **(**Wilmington, MA) served as subjects. Rats were housed individually with the exception of two that were housed together due to space limitations. A continuous 12/12 light/dark cycle was arranged in the colony room and water was available ad libidum in all home cages and within the experimental chambers. Nutritional grain-based rat pellets (45 mg; Bio-Serv, Frenchtown, NJ) earned during experimental sessions were the only available source of food (i.e., no supplemental feedings were provided for the duration of the experiment) as is characteristic of a closed economy. Weights were recorded daily to ensure subjects maintained at least 85% ad libidum weight throughout the experiment. All animal use was in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of Kansas.

Apparatus

Sessions took place within standard operant chambers (24.1 cm x 30.5 cm x 21.0 cm; Med Associates, Inc., St. Albans, VT). Centered on the front wall of each chamber and positioned 1 cm above the floor grid was a pellet receptacle (3 cm x 4 cm) into which a pellet dispenser (Coulbourn Instruments, Allentown, PA) could deliver 45-mg food pellets. Above the receptacle (10 cm) was a non-retractable lever with retractable levers to the left and right (spaced 11 cm apart). A 28-volt DC shielded stimulus lamp was positioned 6 cm above each lever. A house light was centered 19 cm above the floor on the rear wall. Each chamber was equipped with a

white noise speaker and was situated within a sound-attenuating box (Med Associates, Inc.). All experimental events were programmed using MED-PC software and were executed via a PC in an adjacent room.

Procedure

Prior to the first experimental session, reliable responding on all three levers was established using an autoshaping procedure. Following autoshaping, rats completed sessions in which they were able to choose between obtaining food from a predictable food source (fixed number of lever presses required to obtain food) or from a "gambling" food source (unpredictable number of lever presses required per food reinforcer). Each experimental session began with a series of four 21-trial blocks. The first 16 trials in each block were forced-choice trials designed to expose the rat to the contingencies of reinforcement arranged on the gambling and non-gambling alternatives before free choices would be made. The first 8 forced-choice trials were completed on one lever (randomly selected) and the remaining 8 trials were completed on the other lever. These forced-choice trials permitted ample exposure to the four possible response requirements comprising the gambling alternative (discussed below). The lever to which the gambling alternative was assigned was counterbalanced across subjects.

On forced-choice trials, a single response on the center lever extinguished the lamp above the center lever and lit the lamp above one of the two side levers which was inserted into the chamber. If the gambling lever was inserted, the rat was required to complete a variable-ratio (VR) schedule requirement on that lever to obtain three

food pellets. The VR requirement was completed when either 1, 33, 67, or 99 responses were emitted. Between trials, the VR response requirement was randomly determined with replacement. In this way, VR response requirements were unpredictable and "gambling-like," approximating the unpredictability of the number of wagers that must be placed before a win in the human gambling milieu.

If the non-gambling lever was inserted on a forced-choice trial, the rat was required to complete a fixed-ratio (FR) schedule requirement to earn three food pellets. Unlike the VR response requirements, the number of responses required per food pellet was the same every time pellets were obtained. In this way, the FR response requirement was designed to simulate non-gambling sources of human income (e.g., predictable income earned upon completion of a job).

To enhance discrimination between the different contingencies arranged on the two levers, for 4 of the rats the stimulus lamp above the gambling alternative flashed at a 0.5-second frequency, while the stimulus lamp for the non-gambling alternative remained constantly illuminated. For the other 3 rats, this stimulus pairing was reversed. Once the response requirement was completed, the stimulus lamp and house light were immediately extinguished, the side lever retracted, and three food pellets were delivered, each of which was accompanied by a 0.5-second flash of light from within the pellet receptacle. The next trial was initiated immediately after the last pellet was delivered.

The five remaining trials within each of the four 21-trial blocks were free-choice trials. On free-choice trials after the rat pressed the center lever, the stimulus

lamps above both side levers were lit (one flashing). To allow adequate exposure to these stimuli, the rat was then required to emit four additional responses on the center lever before the center lamp was extinguished and both side levers were inserted into the chamber. On free-choice trials a single response on a side lever retracted the other lever and extinguished its stimulus lamp for the remainder of the trial. Once the scheduled contingency on the operative lever was satisfied, the stimulus lamp and house light were extinguished, the lever was retracted, and three food pellets were delivered as in forced-choice trials. The next trial was initiated immediately after the last pellet was delivered. After four of these 21-trial blocks (84 trials), the remainder of the session was composed of free-choice trials. Sessions continued until the rat had expended its response budget (see below) or 4 hours elapsed, whichever came first.

Response budget. Responses allocated to either side lever on the free-choice trials were subtracted from a daily fixed response budget. Responses emitted on forced-choice trials were not counted against the response budget. When the rat exhausted its response budget, the session ended – all lights in the chamber were extinguished and side levers were retracted.

The daily response budget was arranged such that exclusive preference for the FR alternative always resulted in the maximum amount of food obtainable (121 reinforcer events) and any free choices allocated to the VR alternative resulted in relatively less income. This was because the average VR value was always higher than that arranged on the FR lever. Thus, allocating responses to the VR alternative

reduced within-session food earnings when compared to what could have been obtained had the FR alternative been exclusively selected.

Low-gambling baseline. Rats were randomly assigned to begin the study in conditions arranged to produce either low or high preference for the gambling (VR) alternative. Low preference for gambling was defined as 20% or fewer free-choices on the gambling lever. Pilot research indicated an FR requirement of 5 responses (FR-5) produced low preference for gambling, so FR-5 was initially programmed. If a rat's percent choice for the gambling alternative exceeded 20% for two consecutive sessions, the FR requirement was decreased until percent VR choice was below the 20% criterion; these adjustments occurred in 3 of 7 rats (see Table 1). One rat (Red 2) chose the VR alternative on more than 20% of the free choice trials even at FR-2. For this rat, FR-2 served as the terminal requirement on the non-gambling alternative.

After ten consecutive sessions in which VR choice was less than 20%, and the FR value was not adjusted across those sessions, the pre-session drug-administration regimen was initiated (see below). At the conclusion of this condition, rats that completed the low-gambling baseline first completed the high-gambling baseline next. Subjects that completed the high-gambling baseline prior to the low-gambling baseline (opposite order) finished the study upon completing this condition.

High-gambling baseline. In addition to the low-gambling baseline, a baseline of high-gambling preference was examined to address the possibility that increases in percent VR choice in the low-gambling baseline might be explained by non-specific drug effects (e.g., compromised discrimination). Decreases in percent VR choice in

the high-gambling baseline, symmetrical to those seen in the low-gambling baseline, would suggest these non-specific effects.

To produce high-gambling preference, pilot data suggested that an FR-40 would yield ≥80% choice of the VR alternative on free-choice trials. In all cases, rats met this high-gambling criterion at FR-40. Ten consecutive sessions within this range, with no adjustments to the FR value, satisfied the stability criterion and initiated the pre-session drug-administration regimen.

At the conclusion of this condition, rats completing this high-gambling baseline first then completed the low-gambling baseline. Rats that completed the low-gambling baseline prior to the high-gambling baseline (opposite order) finished the study upon completing this condition.

Drug administration. Pramipexole (*N*'-propyl-4,5,6,7-tetrahydrobenzothiazole-2,6-diamine dihydrochloride) was synthesized and provided by Drs. Shaomeng Wang and Jianyong Chen (University of Michigan, Ann Arbor, MI). Pramipexole was dissolved in physiological saline, refrigerated, and protected from light. Four doses of pramipexole (0.03, 0.1, 0.18, 0.3) or saline vehicle were administered subcutaneously 10 min prior to the session at a volume of 1.0 ml/kg (Collins et al., 2005).

Once a stable low- or high-gambling baseline was established, an initial saline dose was administered. Pramipexole was then administered in a descending dose order as previous work in the Fowler lab with dopamine agonists has indicated that the effects of smaller doses may change after larger doses are administered, and so

beginning the sequence with the larger dose may minimize variability. Each saline or drug administration was separated by at least four no-injection sessions (median: 4; range: 4-22 days). These sessions continued until choice returned to the baseline range for four consecutive sessions. After each dose in the descending series was tested, the dosing regimen was repeated two times, each time separated by a saline session. Again, after all doses had been administered in either the low- or high-gambling baseline, the rat completed three series of pre-session pramipexole administrations in the remaining baseline condition.

Data analysis. The primary dependent measure of interest was percent choice for the gambling-like, VR alternative. Data from low- and high-gambling baselines were analyzed using separate two-way repeated-measures analysis of variance (ANOVA; SPSS 16.0, SPSS Inc., Chicago, IL) with "dose" (saline, 0.03, 0.1, 0.18, 0.3) and "series" (first, second, and third) as within-subject factors. One rat (Blue 4) failed to complete any free-choice trials across two sessions at the 0.3 mg/kg dose. This single piece of missing data was replaced by the across-subjects mean percent VR choice at that dose. A paired-samples t-test was used to test for differences between VR values obtained from drug sessions in low- and high-gambling baselines.

Because of the known motor-impairing effects of pramipexole (Lagos et al., 1998), median latencies to respond on all three levers (center, FR, and VR) were recorded. To facilitate comparison of latencies from low- and high-gambling baselines, differences between control (no injection) and drug session latencies were computed and subjected to three-way repeated-measures ANOVAs with "dose,"

"series," and "baseline" (low- or high-gambling) as within-subject factors. In the cases of median FR and VR latencies, only data from forced-choice trials were used in these analyses because subjects occasionally exhibited exclusive choice for a single alternative in all free-choice trials during a session.

Lastly, because recent research detected D_2/D_3 agonist-related increases in perseverative responding (Boulougouris, Castañé, & Robbins, 2009), conditional probabilities of same-lever choice in transitions between forced- and free-choice trials were calculated and analyzed using three-way ANOVA. On occasions where distributions violated assumptions of sphericity and were not amenable to transformations, *F* statistics were compared to critical values calculated using Greenhouse-Geisser adjusted degrees of freedom.

Results

Results of a paired-samples *t*-test indicated VR values obtained in the drug sessions did not differ significantly across low- and high-gambling baseline conditions ($t(104) = 1.08$, $p = .28$).

Figure 1 shows group and individual mean percent VR choice ($\pm$ SEM) as a function of pramipexole dose (see Table 1 in Appendix A). In the low-gambling baseline, represented by the open symbols, pramipexole significantly increased percent VR choice relative to saline levels (significant main effect of dose: $F(4, 24) = 6.94$, $p = .001$, $\eta_p^2 = .54$), but the effect was not dose related (linear contrast: $p = .063$). An effect of dosing series (i.e., first, second, or third exposure to saline and active doses) was observed only at the 0.3 mg/kg dose (Bonferroni corrected $p < .02$).

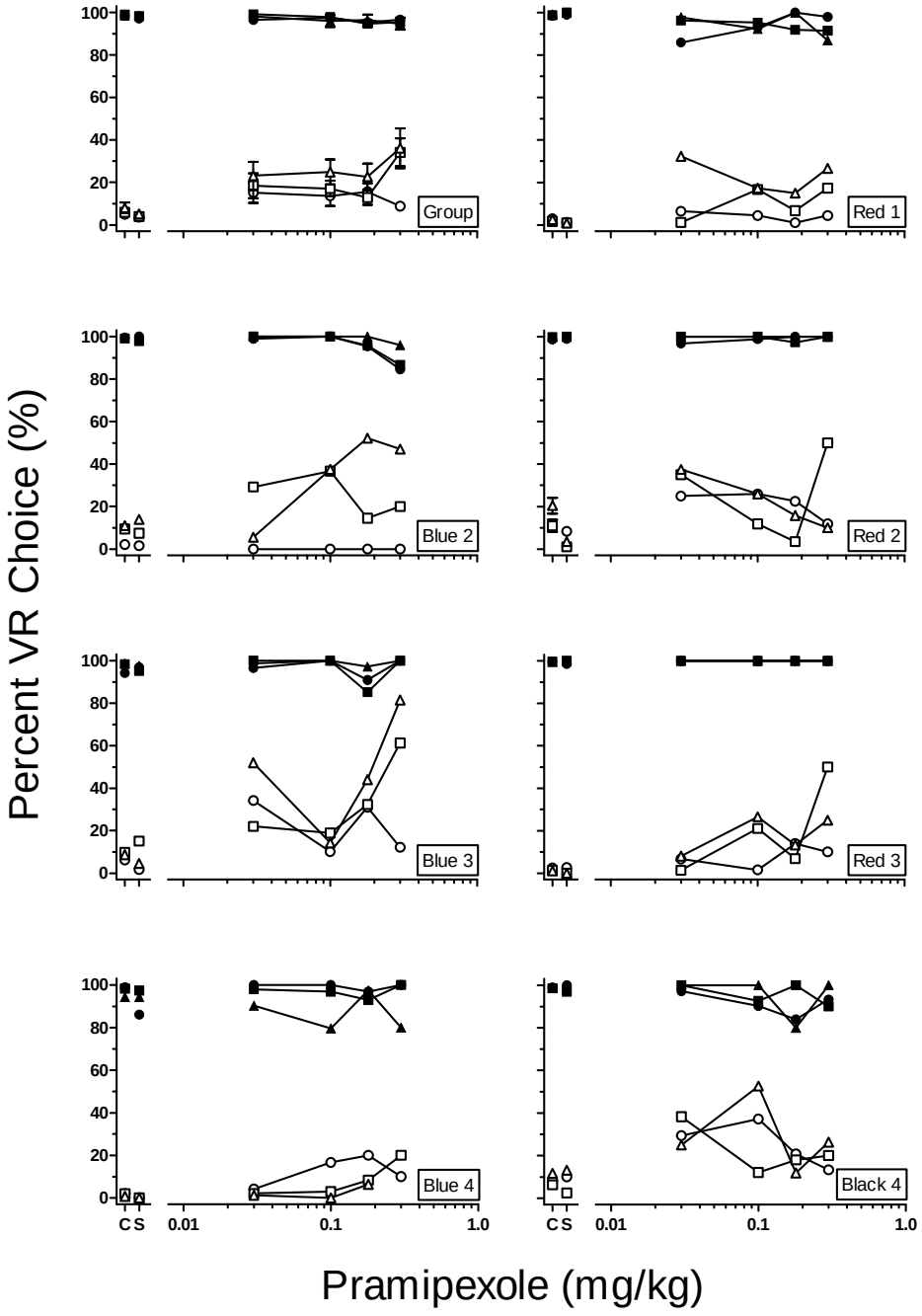

Figure 1. Mean percent VR choice as a function of pramipexole dose in low- (○) and high-gambling (●) baselines. "C" and "S" represent control (no injection) and saline, respectively. Circles, squares, and triangles represent dosing series 1-3, respectively.

This sensitization effect was observed between the first (circles) and second (squares) series of doses.

Also shown in Figure 1 are data from the high-gambling baseline (see Table 2 in Appendix A). In this baseline (filled symbols), percent VR choice in drug sessions was not significantly different from saline sessions ($p = .33$), and there was no significant main effect of series ($p = .82$). As stated previously, this baseline acted as a control condition for the possibility that pramipexole-related increases in the low-gambling baseline could be alternatively explained by non-specific drug effects such as disrupted discrimination between response alternatives. If this were the case, percent VR choice in the high-gambling baseline would likely have decreased in a manner opposite the increases seen in the low-gambling baseline. This effect is absent in Figure 1, suggesting that non-specific effects likely did not account for the increases in percent VR choice observed in the low-gambling baseline.

Figure 2 shows median latencies to respond on the center lever, expressed as a difference from control (no injection) center-lever latencies. As is evident in the grouped data, latency differences from control tended to increase as a function of dose (significant main effect of dose: $F(4, 24) = 25.45$, $p < .001$, $\eta_p^2 = .81$; significant linear contrast: $p = .02$). None of the interactions involving dose were significant (p's $\geq .09$). Latency differences depended upon the dosing series and baseline condition (series x baseline interaction: $F(2,12) = 6.99$, $p = .01$, $\eta_p^2 = .54$). Follow-up ANOVAs were conducted for each baseline and revealed that only in the high-gambling baseline did the dosing series interact with drug dose to affect the magnitude of the

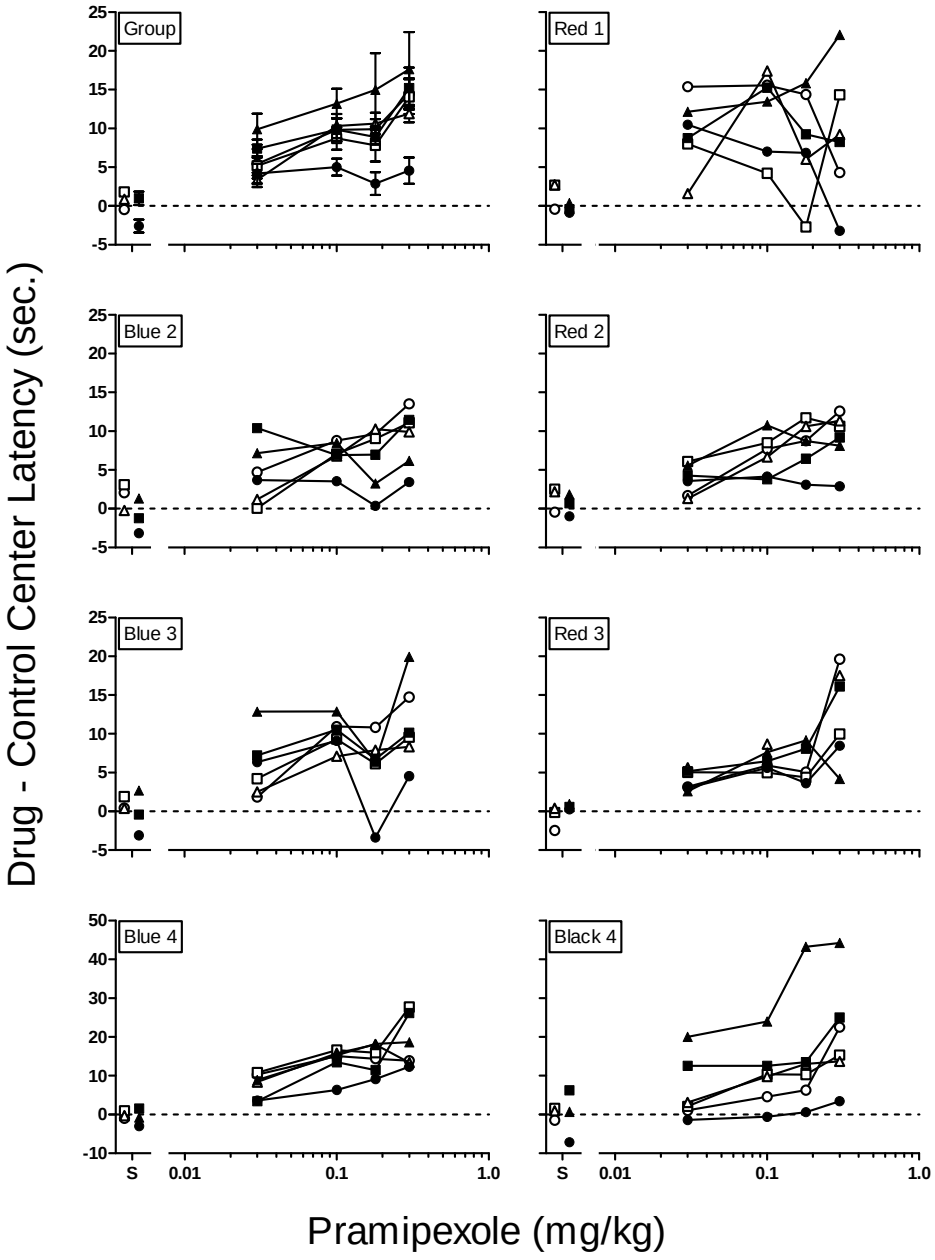

Figure 2. Difference between median drug and control center lever latencies as a function of pramipexole dose in low- (○) and high-gambling (●) baselines. Circles, squares, and triangles represent dosing series 1-3, respectively. Note y-axis scales for Blue 4 and Black 4.

latency difference (significant series x dose interaction: $F(8,48) = 35.3$, $p < .04$, $\eta_p^2 = .27$), suggesting baseline-dependent sensitization to repeated drug exposures.

Figure 3 shows median latencies to make the first response on the FR lever on forced-FR trials, expressed as a difference from control (no injection) FR latencies. As in the previous figures, latencies are separated by baseline and dosing series. Latencies in the high-gambling baseline were significantly longer than those in the low-gambling baseline (main effect of baseline: $F(1, 6) = 50.42$, $p < .01$, $\eta_p^2 = .83$). A significant dose x baseline interaction was also detected ($F(4, 24) = 3.78$, $p < .02$, $\eta_p^2 = .39$). Further investigation of this interaction using a paired samples *t*-test revealed significant differences in latency differences between the two baselines at all active doses (all p's $\leq .001$). Latency differences in saline sessions were not different across low- and high-gambling baselines ($t(20) = .395$, $p = .7$).

Figure 4 shows differences between median drug and control VR lever latencies for both baselines. Median VR latencies were brief compared to latencies on the center and FR levers (compare Figs. 3 and 4). Because no main effect was evident for series and it did not interact with other variables, data shown in Figure 4 were collapsed across this factor. Significant main effects of dose ($F(4,80) = 7.8$, $p < .001$, $\eta_p^2 = .28$) and baseline ($F(1,20) = 7.8$, $p = .013$, $\eta_p^2 = .27$) were observed; however, there was no significant dose x baseline interaction ($p = .11$). Post-hoc Bonferonni-corrected comparisons revealed significantly smaller differences between control and drug VR latencies in the high-gambling baseline compared with latencies in the low-gambling baseline (a difference of 0.1 sec).

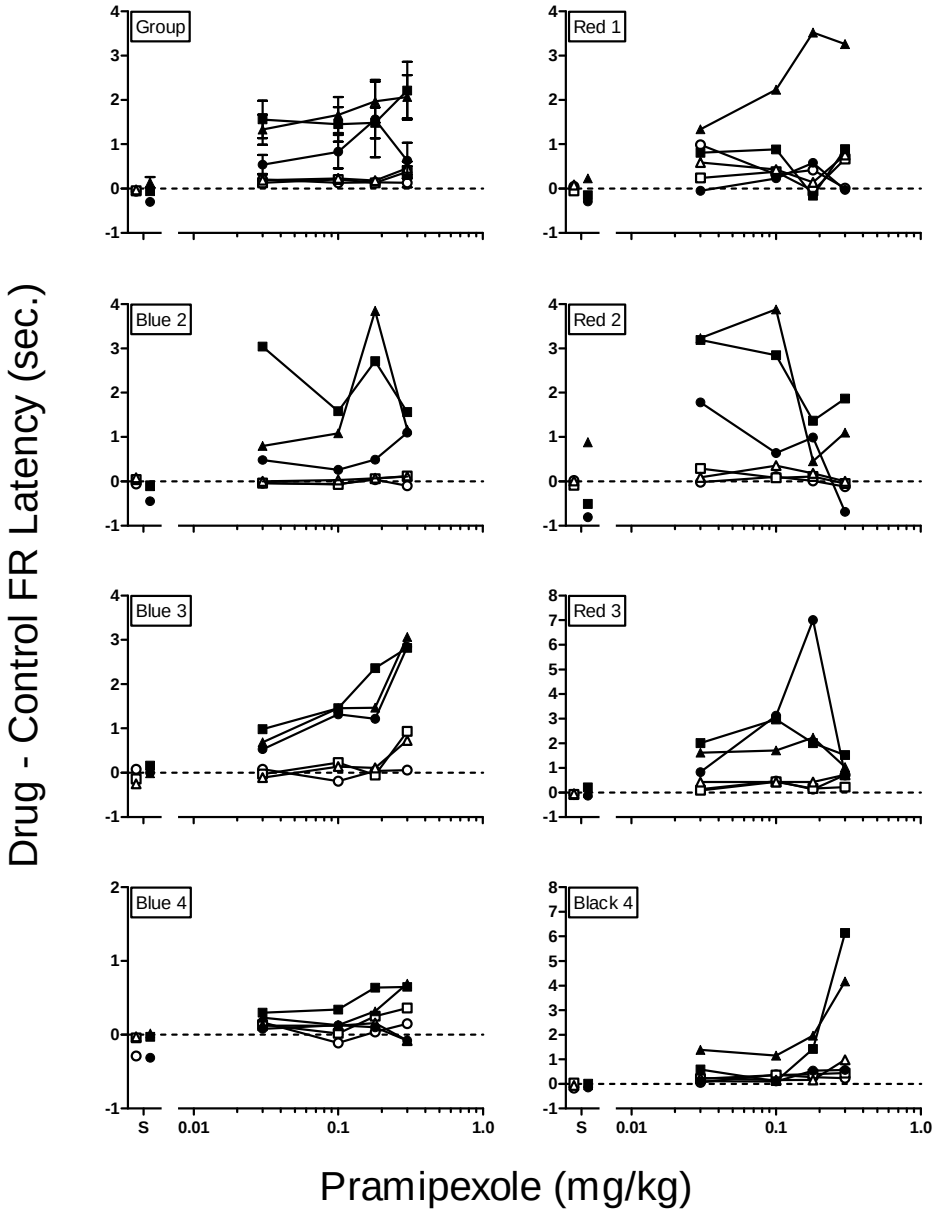

Figure 3. Difference between median drug and control FR lever latencies as a function of pramipexole dose in low- (○) and high-gambling (●) baselines. Circles, squares, and triangles represent dosing series 1-3, respectively. Note individually scaled y-axes.

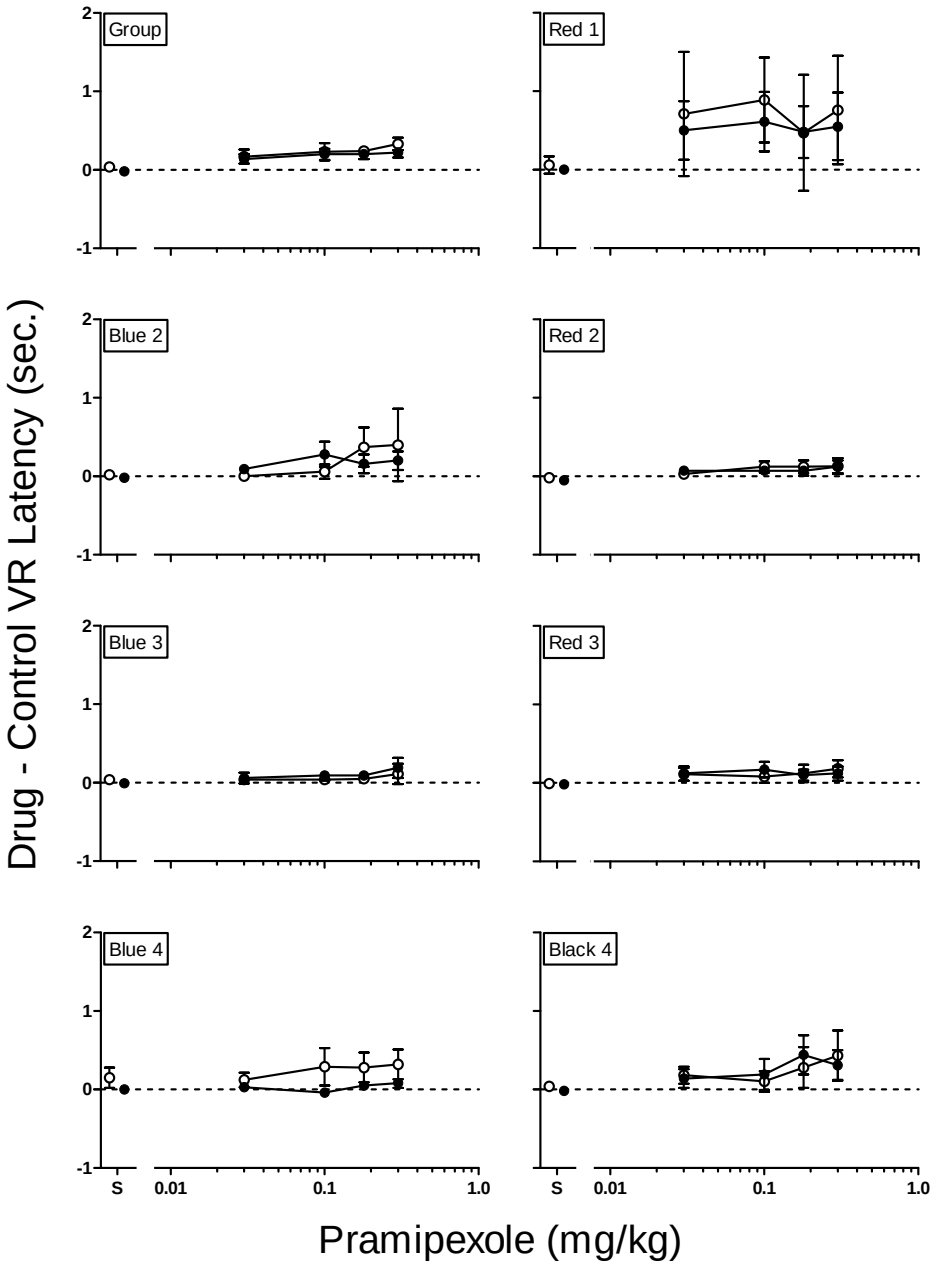

Figure 4. Difference between median drug and control VR lever latencies as a function of pramipexole dose in low- (○) and high-gambling (●) baselines. "C" and "S" represent control (no injection) and saline, respectively. Error bars are SEM.

To investigate the perseverative effects of pramipexole (e.g., Boulougouris et al., 2009), the conditional probability of making the first free-choice on the lever that ended the series of forced-choice trials was calculated at each dose. Specifically, transitions in which subjects chose the alternative (i.e., during a free-choice trial) that had been presented in the immediately preceding forced-choice trial (i.e., staying) were scored as positive instances. Choices for the alternative opposite the one forced in the immediately preceding trial (i.e., switching) received neutral scores. The sum of these instances divided by the total number of forced-free transitions within a session (the maximum was 4) permitted a measure of response perseveration.

Figure 5 shows mean conditional probabilities of the first free-choice occurring for the recently-forced lever in both low- and high-gambling baselines. Probabilities were unaffected by baseline condition ($p > .8$). Significant main effects of dose ($F(4, 52) = 2.74$, $p < .04$, $\eta_p^2 = .17$) and of series ($F(2, 26) = 5.71$, $p < .01$, $\eta_p^2 = .31$) were detected. The only significant differences identified by post-hoc Bonferroni-corrected pairwise comparisons were those between the mean conditional probabilities at saline and 0.18 mg/kg ($p = .03$). Interestingly, mean conditional probabilities of staying at a recently-forced alternative were highest in saline sessions (mean = .57) and lowest in sessions in which 0.18 mg/kg was administered (mean = .45), indicating an increased probability of switching to the alternative *opposite* to the one that had been recently forced. Conditional probabilities in sessions in which smaller doses were administered were more similar to saline probabilities, suggesting a quadratic trend in the function ($p < .02$). Results of pairwise comparisons of the

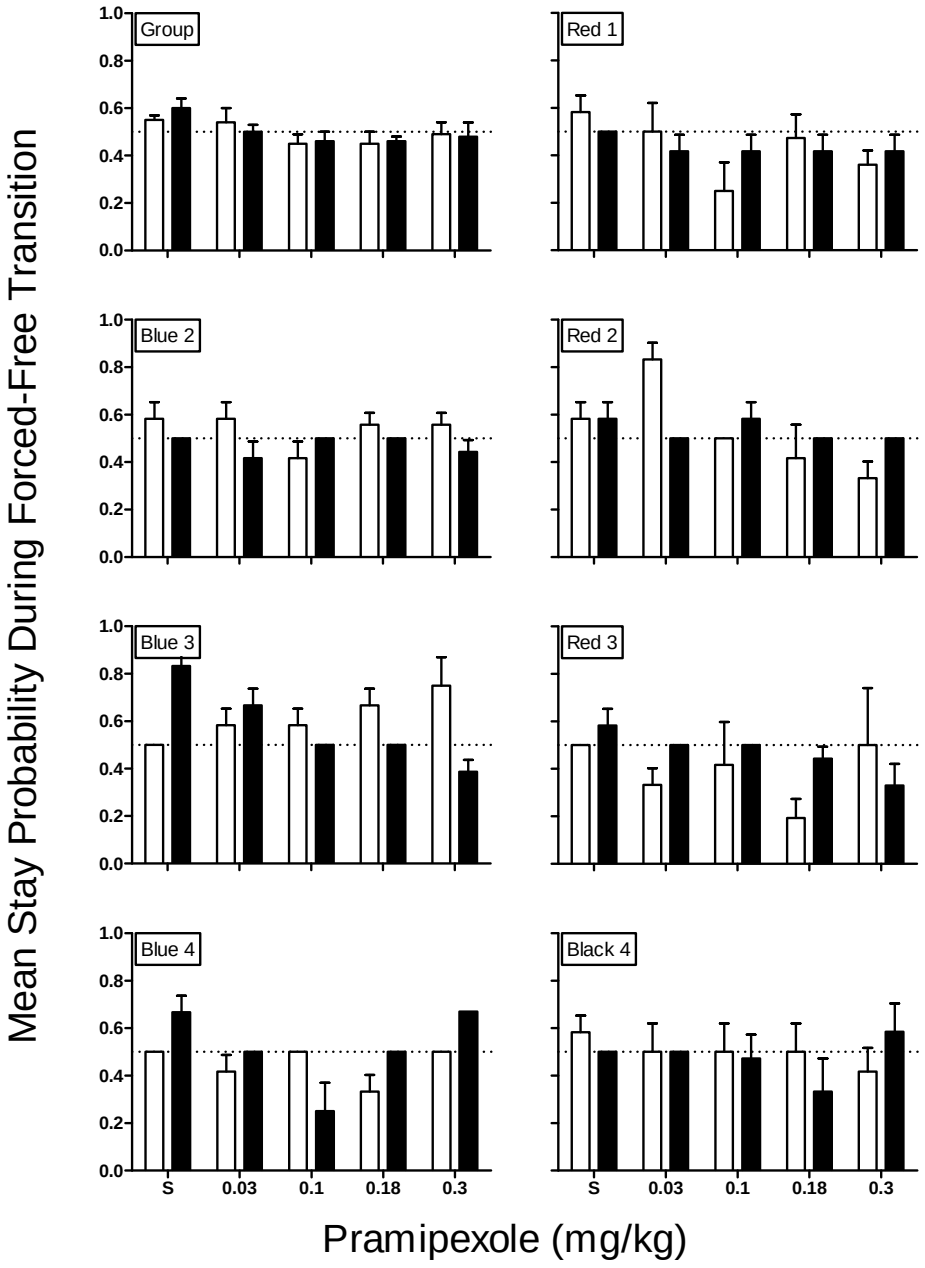

Figure 5. Individual and group conditional probabilities of making the first free-choice on the lever that ended the series of forced-choice trials in low- (□) and high-gambling (■) baselines. Error bars are SEM.

factor level means for series also indicated a decreasing linear trend in the probability of same-lever responding with repeated administrations (p = .02).

Discussion

In the present study, an animal model of gambling was used to determine the effects of pramipexole, a D_2/D_3 dopamine agonist, on preference for gambling-like sources of reinforcement. Of primary importance is the finding that acute administration of pramipexole was observed to modestly increase rats' preferences for food reinforcement obtained from a gambling-like alternative relative to saline levels. When pramipexole was administered under baseline conditions of high-gambling preference, the drug did not significantly affect choice for the gambling-like alternative. This pattern of results appears consistent with the clinically documented coinciding events of emergent pathological gambling and initiation of pramipexole and other dopamine agonist regimens (Crockford et al., 2008). Further, because preference was affected solely in the low-gambling baseline, additional explanations for the present findings, such as compromised discrimination between the two response alternatives, can be tentatively ruled out.

In contrast, non-specific drug effects were unlikely completely absent in the present study. Consistent with previous findings that pramipexole (Lagos et al., 1998) and other D_3-preferring agonists (Ouagazzal & Creese, 2000) suppress locomotor activity, pramipexole increased latencies to initiate new trials (center lever) and to begin responding on the FR or VR alternatives (forced-choice only) when compared to latencies from control sessions. Although generalizing across species is difficult,

human simple and procedural reaction times also increase as a function of acute pramipexole dose (Hamidovic et al., 2008; Pizzagalli et al., 2008), suggesting a common effect of D_3-preferring medications to decrease motor activity.

Given recent evidence that quinpirole, a D_2/D_3 dopamine agonist like pramipexole (but with lower relative preference for D_3 to D_2 receptors), inhibits reversal learning (i.e., promotes response perseveration) (Boulougouris et al., 2009), we speculated that in drug sessions rats would be more likely to choose an alternative in a free-choice trial that had been the forced-choice in the immediately preceding trial. This hypothesis was not supported. Indeed, the conditional probability of a perseverative free-choice actually decreased during pramipexole sessions when compared to saline sessions.

Together, these findings lend credence to a putative link between pramipexole and impulsive behavior identified by the existing clinical literature. More importantly, the results of the present study provide a systematic extension of previous experimental investigations related to pramipexole and other D_2/D_3 agonist-induced behavioral perturbations in healthy subjects (e.g., Hamidovic et al., 2008; Riba et al., 2008). Unlike these previous studies, the present experiment and others conducted in our laboratory (e.g., Madden et al., in preparation) demonstrate the viability of nonhuman models of impulsive decision-making and their ability to characterize within-subject drug effects in a population whose historical experiences are well-described and controlled. Along with these studies, the preliminary interpretations that follow take into account current neurobehavioral research regarding the influence

of endogenous dopamine and dopaminergic medications on brain activation in areas correlated with impulsive or risky decision-making. More specifically, the disruptive effects of pramipexole on reward- and punishment-related learning processes and their implications for the present findings are discussed.

Dopaminergic Participation in Dysfunctional Learning

Through the use of neuroimaging technologies, researchers have recently identified brain regions involved in aspects of reward-related learning. Of note are midbrain structures such as the ventral striatum, which is innervated by dopamine and characterized by a relative density of the D_3 receptor subtype compared to other brain regions. Reuter et al. (2005), using fMRI technology, reported that individuals diagnosed with pathological gambling had decreased activation in the right ventral striatum relative to matched controls while completing a gambling task. Further, striatal activation within the sample of pathological gamblers was negatively correlated with gambling severity. On the whole, this reduced activation is similar to that found in drug-addicted individuals (see Volkow, Fowler, Wang, & Goldstein, 2002 for a review). Beyond these similarities in brain activity, however, it is currently unknown whether diminished activation in these areas is the result of addiction or whether lower levels of striatal activity selectively predispose individuals to engage in impulsive behavior such as drug-taking or gambling.

When coupled with dopamine agonists, hypoactivation of the striatum is strongly correlated with risky decision-making. Recent studies have shown that dopamine agonist medications like pramipexole, while enhancing dopaminergic tone

in general, may reduce activation along mesolimbic pathways and disrupt processes essential to reward-related learning and reward prediction. For instance, fMRI measures suggest acute pramipexole blunts responding along the cortical loop containing the striatum and other structures (Riba et al., 2008). Moreover, these authors reported that this hypoactivation was accompanied by an increased likelihood to choose a less conservative option in a lottery-style gambling task when compared with choices the same human participants made during placebo sessions. Likewise, Pizzagalli et al. (2008) found a single acute dose of pramipexole (0.5 mg) disrupted the ability of healthy human volunteers to learn to select a more frequently rewarded alternative during a probabilistic differential reinforcement task relative to a control group receiving a placebo. However, individuals receiving chronic treatment with dopamine agonists such as pramipexole, ropinirole, and cabergoline (average 0.47 mg/dose pramipexole equivalent) exhibit greater activation of the mesolimbic reward system (e.g., ventral striatum, orbitofrontal cortex) relative to their "off-DA" performance during a probabilistic monetary task (Abler, Hahlbrock, Unrath, Grön, & Kassubek, 2009). Despite this absence of agonist-related reductions in brain activation when agonists are taken chronically, Abler et al.'s human participants displayed reward prediction signaling contrary to that observed in seminal work by Schultz and colleagues with nonhuman primates (Fiorillo, Tobler, & Schultz, 2003; Schultz, 1998; Waelti, Dickinson, & Schultz, 2001). Although these findings collectively demonstrate the potential for pramipexole and other dopamine agonists to impair learning processes in novel tasks, the question of why steady-state preference

was affected in the present study remains unanswered. Nonetheless, the studies reviewed above suggest a critical role for dopamine and dopamine agonists in reward processing.

In the present study, acute pramipexole increased rats' preferences for gambling-like sources of reinforcement. The results of the aforementioned studies suggest two interpretations. First, pramipexole may enhance an organism's sensitivity to the unpredictable scheduling of rewards operating on gambling-like schedules. Evidence for this sensitization effect comes from work by Fiorillo et al. (2003). They reported that the activity of dopamine neurons in the ventral striatum peaked and sustained under conditions of maximum uncertainty (i.e., reward probability = 0.5). If pramipexole decreases activation of dopaminergic neurons in regions specific to reward valuation and learning, then engagement in unpredictable and impulsive courses of action may induce supplementary dopamine transmission. In other words, impulsive behavior observed under dopamine agonists may represent compensatory efforts on the part of the individual to regulate striatal dopamine concentrations (e.g., Riba et al., 2008).

Second, pramipexole may desensitize an organism to the effects of aversive outcomes, such as the income loss experienced by our rats in sessions in which they chose the "higher-priced" variable-ratio alternative. The interpretation that pramipexole reduces the psychological effects of aversive outcomes is partially supported by the results of a recent study by Bódi et al. (2009). In their study, DA-agonist medicated and never-medicated young onset PD patients were tested on

feedback-based probabilistic classification tasks (e.g., card-sorting) and measures of novelty-seeking. In the feedback-based task, participants were asked to assign stimuli to one of two classes (A or B) depending upon feedback from previous trials involving differential rewards (80 trials) and punishments (80 trials) contingent upon correct or incorrect answers, respectively. Across four blocks of 20 trials in the reward-learning phase, PD patients receiving dopamine agonists (i.e., pramipexole [mean dose: 4.5 mg/day] or ropinirole [mean dose: 5.5 mg/day]) showed optimal learning consistent with matched, non-PD controls, whereas performance of never-medicated PD patients revealed decision-making only slightly higher than chance levels. In the punishment-learning phase, the opposite effect occurred: Never-medicated PD patients learned the task as well as the control group, while the D_2/D_3 agonist group demonstrated decision-making consistent with chance. Furthermore, after initiating DA agonist therapy, the previously never-medicated group showed improvements in reward-related learning and deficits in punishment-related learning similar to those observed in the original DA-agonist medicated group. On the whole, participants in the Bódi et al. study receiving DA-agonist treatment showed few, if any, deficits in reward learning, while at the same time demonstrating a gross inability to modulate their choices in the face of punishing consequences. Applied to the context of the present study, Bódi et al.'s (2009) findings provide a potential explanation of pramipexole-induced increased preference for gambling-like schedules of reinforcement in terms of reduced sensitivity to aversive outcomes in the form of global reductions in food income. Likely also is the possibility that pramipexole

sensitizes an organism to dopaminergic increases resulting from unpredictable reward scheduling. While these interpretations are by no means exclusive, further research into the effects of pramipexole on reward- and punishment-learning is necessary to elaborate their contributions to the present findings.

A few limitations of this research deserve comment. First, the function of the programmed response budget remains unclear. For example, whether the rats' behavior was sensitive to the molar consequences of income lost, and further, whether these losses were perceived as being contingent upon responses allocated to the more costly VR alternative is difficult to distinguish. Also, because the response budget was derived from the FR value in order to hold maximum reinforcement obtained constant across low- and high-gambling baselines, even a modest number of VR choices in the low-gambling baseline subtracted disproportionately more from the relatively smaller budgets programmed in this condition (e.g., 363 for FR-3). Therefore, if the rats were indeed sensitive to these contingencies, then the lower response budgets in the low-gambling baseline may have actually resulted in lower VR choice in drug sessions than would have otherwise been observed.

To address this hypothesis, the present study has been systematically replicated with the exception of the response budget in seven experimentally naïve subjects. The results of the present work were partially reproduced: The same acute pramipexole doses increased VR choice in a manner similar to that seen here, although the magnitude of these increases at lower doses (e.g., 0.03 & 0.1 mg/kg) was substantially reduced (data not shown here). Statistical tests (ANOVA) confirmed VR

choice did not differ significantly between the two studies (p = .08), although the dose x experiment interaction did trend toward significance (p = .058), likely due to the comparatively smaller effects of lower pramipexole doses in the follow-up study. Thus, the response budget may not constitute an important variable in our conceptualization of human gambling and in determining pramipexole's capacity to induce impulsive behavior.

A second limitation is that we did not use Parkinsonian rats. An argument could be made that because individuals diagnosed with PD already exhibit common neurological markers of addiction (i.e., reduced striatal activity), their predisposition to behave impulsively is amplified by dopamine agonist medications, and this interaction does not occur in healthy subjects with intact brain function. Future research should investigate the effects of such dopamine agonists in Parkinsonian animal models. Given these questions, it is clear that the procedures used in the present research require ongoing improvement.

Despite these limitations, the present study detected measurable increases in gambling-like behavior when pramipexole was acutely administered. Whether these effects are explicable in terms of deficient reward processing, hypersensitivity to unpredictable rewards, or marked insensitivity to aversive outcomes (or all of these interpretations simultaneously) is unknown. The findings and procedures incorporated herein call for further explication and refinement. We are confident, however, that the relation between impulsive behavior in the form of pathological gambling and pramipexole, as well as other D_2/D_3 dopamine agonists with similar

affinities, will comprise an increasingly relevant line of inquiry as it pertains to human addictive disorders.

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List of Appendices

Appendix A

Table 1. Mean percent VR choice; median center, FR, and VR latencies; and mean conditional probability of same-lever choice during forced-free transitions in control, saline, and drug sessions in the low-gambling baseline. SEM are in parentheses.

				Latency			
Rat	FR/ Budget	Dose (mg/kg)	Percent VR Choice	Center	FR	VR	Conditional Probability
Black 4	3/363	Control	8.73 (0.66)	9.68	1.23	0.86	
		Saline	8.53 (2.60)	10.60	1.17	0.90	0.58 (0.07)
		0.03	30.88 (3.18)	11.75	1.44	1.04	0.50 (0.12)
		0.1	33.95 (9.65)	19.43	1.59	0.96	0.50 (0.12)
		0.18	16.83 (2.13)	19.96	1.50	1.14	0.50 (0.12)
		0.3	19.88 (3.06)	25.01	1.67	1.29	0.42 (0.11)
Blue 2	5/605	Control	7.50 (0.64)	6.00	1.09	0.89	
		Saline	7.60 (2.91)	8.06	1.13	0.91	0.58 (0.07)
		0.03	11.58 (7.30)	7.20	1.05	0.89	0.58 (0.07)
		0.1	24.70 (10.08)	13.06	1.03	0.95	0.42 (0.07)
		0.18	22.22 (12.70)	15.64	1.15	1.26	0.56 (0.05)
		0.3	22.35 (11.13)	17.09	1.20	1.29	0.56 (0.05)
Blue 3	5/605	Control	8.24 (0.65)	5.15	1.11	0.73	
		Saline	7.10 (3.33)	5.58	0.97	0.77	0.50 (0.00)
		0.03	36.07 (7.11)	7.65	1.08	0.77	0.58 (0.07)
		0.1	14.43 (2.06)	14.47	1.25	0.77	0.58 (0.07)
		0.18	35.76 (3.38)	13.06	1.15	0.78	0.67 (0.07)
		0.3	51.63 (16.82)	14.70	1.84	0.84	0.75 (0.12)
Blue 4	5/605	Control	1.57 (0.19)	9.91	1.20	1.27	
		Saline	0.00 (0.00)	9.61	1.16	1.42	0.50 (0.00)
		0.03	2.51 (0.73)	20.21	1.34	1.39	0.42 (0.07)
		0.1	6.54 (4.19)	25.69	1.22	1.56	0.50 (0.00)
		0.18	11.59 (3.46)	25.85	1.36	1.55	0.33 (0.07)
		0.3	15.00 (3.54)	23.78	1.35	1.59	0.50 (0.00)
Red 1	3/363	Control	2.27 (0.41)	10.25	1.74	1.84	
		Saline	0.97 (0.06)	12.95	1.79	1.90	0.58 (0.07)
		0.03	13.25 (7.86)	18.26	2.33	2.55	0.50 (0.12)
		0.1	12.75 (3.43)	25.82	2.12	2.73	0.25 (0.12)
		0.18	7.56 (3.32)	16.28	1.88	2.31	0.47 (0.10)
		0.3	16.09 (5.28)	19.47	2.41	2.60	0.36 (0.06)
Red 2	2/242	Control	14.11 (1.76)	7.03	1.17	0.78	
		Saline	4.27 (1.76)	9.22	1.18	0.76	0.58 (0.07)
		0.03	32.50 (3.12)	8.72	1.26	0.81	0.83 (0.07)
		0.1	21.21 (3.86)	14.75	1.27	0.90	0.50 (0.00)
		0.18	13.93 (4.54)	17.65	1.28	0.90	0.42 (0.14)
		0.3	23.92 (10.66)	18.36	1.12	0.91	0.33 (0.07)
Red 3	5/605	Control	1.59 (0.21)	8.68	1.04	0.78	
		Saline	0.87 (0.71)	8.55	0.96	0.77	0.50 (0.00)
		0.03	5.39 (1.67)	13.72	1.18	0.89	0.33 (0.07)
		0.1	16.39 (6.18)	14.60	1.47	0.86	0.42 (0.18)
		0.18	11.38 (1.87)	13.75	1.20	0.90	0.19 (0.08)
		0.3	28.33 (9.53)	26.20	1.75	0.96	0.50 (0.24)
Group		Control	6.29 (1.65)	8.68	1.17	0.86	
		Saline	4.19 (1.26)	9.22	1.16	0.90	0.55 (0.02)
		0.03	18.88 (4.87)	11.75	1.26	0.89	0.54 (0.06)
		0.1	18.57 (3.13)	14.75	1.27	0.95	0.45 (0.04)
		0.18	17.04 (3.32)	16.28	1.28	1.14	0.45 (0.05)
		0.3	25.32 (4.36)	19.47	1.67	1.29	0.49 (0.05)

Table 2. Mean percent VR choice; median center, FR, and VR latencies; and mean conditional probability of same-lever choice during free-forced transitions in control, saline, and drug sessions in the high-gambling baseline. SEM are in parentheses.

				Latency			
Rat	FR/ Budget	Dose (mg/kg)	Percent VR Choice	Center	FR	VR	Conditional Probability
Black 4	40/4840	Control	98.73 (0.14)	23.57	1.20	0.77	
		Saline	98.12 (0.77)	24.24	1.15	0.75	0.50 (0.00)
		0.03	99.06 (0.77)	36.10	1.79	0.91	0.50 (0.00)
		0.1	94.26 (2.41)	36.10	1.31	0.96	0.47 (0.10)
		0.18	88.00 (4.99)	37.02	2.62	1.21	0.33 (0.14)
		0.3	94.44 (2.40)	48.62	5.37	1.08	0.59 (0.05)
Blue 2	40/4840	Control	99.41 (0.13)	11.63	1.61	0.83	
		Saline	99.29 (0.58)	10.38	1.50	0.81	0.50 (0.00)
		0.03	99.66 (0.27)	18.80	2.41	0.92	0.42 (0.07)
		0.1	100.00 (0.00)	18.55	2.69	1.11	0.50 (0.00)
		0.18	97.09 (1.19)	14.86	4.32	0.99	0.50 (0.00)
		0.3	89.10 (2.86)	17.79	2.78	1.03	0.44 (0.05)
Blue 3	40/4840	Control	97.14 (0.42)	11.36	1.01	0.72	
		Saline	96.58 (0.61)	10.95	1.01	0.71	0.83 (0.07)
		0.03	98.44 (0.80)	18.57	1.70	0.78	0.67 (0.07)
		0.1	100.00 (0.00)	21.89	2.47	0.81	0.50 (0.00)
		0.18	91.11 (2.84)	17.78	2.48	0.81	0.50 (0.00)
		0.3	100.00 (0.00)	21.53	3.85	0.91	0.39 (0.05)
Blue 4	40/4840	Control	97.18 (0.57)	8.82	1.17	1.09	
		Saline	92.58 (2.74)	8.05	1.14	1.09	0.67 (0.07)
		0.03	96.06 (2.44)	12.40	1.40	1.12	0.50 (0.00)
		0.1	92.14 (5.18)	22.28	1.30	1.05	0.25 (0.12)
		0.18	95.73 (1.18)	20.25	1.49	1.14	0.50 (0.00)
		0.3	93.33 (5.44)	27.44	1.82	1.17	0.67 (0.00)
Red 1	40/4840	Control	98.81 (0.12)	11.26	1.52	1.34	
		Saline	99.69 (0.25)	10.84	1.37	1.34	0.50 (0.00)
		0.03	93.36 (3.06)	21.75	2.33	1.84	0.42 (0.07)
		0.1	93.57 (2.19)	24.71	2.40	1.95	0.42 (0.07)
		0.18	97.31 (2.19)	20.49	2.10	1.82	0.42 (0.07)
		0.3	92.15 (2.62)	19.50	2.41	1.89	0.42 (0.07)
Red 2	40/4840	Control	99.46 (0.16)	7.37	1.86	0.78	
		Saline	99.67 (0.27)	8.06	1.35	0.73	0.58 (0.07)
		0.03	98.95 (0.86)	11.63	5.05	0.85	0.50 (0.00)
		0.1	99.63 (0.30)	11.50	4.71	0.85	0.58 (0.07)
		0.18	99.12 (0.72)	13.82	2.85	0.85	0.50 (0.00)
		0.3	100.00 (0.00)	15.49	2.96	0.90	0.50 (0.00)
Red 3	40/4840	Control	99.66 (0.09)	9.18	1.35	0.73	
		Saline	99.56 (0.36)	9.73	1.34	0.71	0.58 (0.07)
		0.03	100.00 (0.00)	12.18	2.97	0.85	0.50 (0.00)
		0.1	100.00 (0.00)	15.64	4.31	0.90	0.50 (0.00)
		0.18	100.00 (0.00)	17.26	3.58	0.83	0.44 (0.05)
		0.3	100.00 (0.00)	17.64	2.38	0.85	0.33 (0.00)
Group		Control	98.63 (0.37)	11.26	1.35	0.78	
		Saline	97.93 (0.92)	10.38	1.34	0.75	0.60 (0.04)
		0.03	97.93 (0.84)	18.57	2.33	0.91	0.50 (0.03)
		0.1	97.09 (1.25)	21.89	2.47	0.96	0.46 (0.04)
		0.18	95.48 (1.53)	17.78	2.62	0.99	0.46 (0.02)
		0.3	95.57 (1.56)	19.50	2.78	1.03	0.48 (0.04)