Parallel role for the dopamine D1 receptor in gambling and amphetamine reinforcement in healthy volunteers

Martin H Zack, Daniela S Lobo, Candice Biback, Tim Fang, Kelly Smart, Daniel Tatone, Aditi Kalia, Daniel Digiacomo and James L Kennedy

Abstract

This study investigated the role of dopamine, and specifically the D1 receptor (D1R), in the reinforcing effects of a slot-machine game in healthy volunteers (n=30). To compare gambling and drug effects, subjects received the prototypic psychostimulant drug d-amphetamine (AMPH; 20 mg) in a multi-session, placebo-controlled design. To isolate D1R, half the subjects were pretreated with the preferential D2 receptor antagonist haloperidol (HAL; 3 mg), and the other half with the mixed D1–D2 antagonist fluphenazine (FLU; 3 mg) before the game (Phase I) and AMPH (Phase II). HAL decreased and FLU increased the post-game desire to gamble and post-AMPH desire to take AMPH again, as well as AMPH scale ratings on the Addiction Research Center Inventory after gambling and AMPH. The effects of the antagonists on desire to gamble and to take AMPH again were significantly intercorrelated. HAL increased and FLU decreased the salience of negative affective words on a rapid reading task after both reinforcers. HAL also decreased the salience of gambling words after AMPH. Both reinforcers increased diastolic blood pressure equally under antagonists and placebo. Results indicate that D1R plays a parallel role in the psychostimulant-like, incentive-motivational, and salience-enhancing effects of gambling and AMPH. Moderate D1R activation appears to optimize these effects in healthy subjects.

Keywords

Gambling, D1, amphetamine, reinforcement

Introduction

Pathological gambling (PG) is a serious psychiatric disorder that affects 1–3% of the general population (Subramaniam et al., 2015), and is linked with great individual and social harm (Estevez et al., 2014; Park et al., 2009). Research has found numerous similarities between PG and drug addiction (Leeman and Potenza, 2012). Accordingly, PG was recently reclassified (as Gambling Disorder) with Substance Dependence in the latest version of the diagnostic manual of psychiatry, the DSM-5 (American Psychological Association, 2013).

If PG is an addiction like substance dependence, the processes that mediate the development of substance dependence should also extend to PG. Chronic exposure to addictive substances is necessary, although not sufficient, for the development of substance dependence (in humans) (Volkow and Li, 2005), and substance-induced neuroplasticity mediates this syndrome (Nestler, 2001). Sensitization, or hyper-reactivity to cues for a drug following chronic exposure to it, is a key form of substance-induced neuroplasticity. Addictive substances share the ability to cause acute striatal dopamine (DA) release. However, DA release is only necessary for the sensitizing effects of amphetamine (AMPH; Vanderschuren and Kalivas, 2000). Activation of DA D1 receptors (D1Rs) in particular appears to be needed both for the induction of sensitization and for the occurrence of AMPH self-administration (i.e., reinforcing effects of the drug; Pierre and Vezina, 1998; Vezina, 1996).

Individuals with PG exhibit increased striatal DA release in response to AMPH (Boileau et al., 2014). Healthy rats repeatedly exposed to reward (sweet solutions) under conditions that mimic the uncertain reinforcement schedules of gambling subsequently displayed increased locomotor response to an acute AMPH challenge and were more prone to work for AMPH (both indicators of DA activation) than animals that received equivalent reward in the absence of uncertainty (Mascia et al., 2015; Singer et al., 2012; Zack et al., 2014). Collectively, these data suggest that DA hyper-reactivity to AMPH in humans with PG may partly reflect sensitization induced by chronic exposure to gambling. That is, gambling may be a causal agent of PG, much like addictive drugs are causal agents of substance dependence.

An episode of slot-machine gambling causes striatal DA release in humans without PG (Joutsa et al., 2012). However, whether gambling specifically activates D1R in healthy humans is unclear. Evidence for such an effect would indicate that gambling engages a critical substrate for AMPH-induced neuroplasticity.

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and that hyper-reactivity to AMPH in humans and animals chronically exposed to gambling-like rewards reflects a parallel sensitizing process to that induced by AMPH itself. The present study was designed to investigate this issue. Specifically, we sought to determine if pharmacological manipulation of DA has a parallel effect on the reinforcing properties of a slot-machine game and a dose of AMPH in healthy volunteers, and if so, to what extent D1R mediates this effect.

Previous research employed a dual-antagonist pretreatment strategy to isolate the role of D1R in AMPH reinforcement in healthy volunteers (Brauer and De Wit, 1995). A low dose (2 mg) of the selective D2R antagonist pimozide decreased the reinforcing effects of a moderate dose of AMPH (20 mg). In contrast, the mixed D1–D2 antagonist fluphenazine (FLU; 3 mg) led to a modest, though nonsignificant, increase in subjective reinforcing and psychomotor facilitating effects of AMPH (20 mg). By removing negative feedback inhibition at D2R autoreceptors, a selective D2R antagonist increases pre-synaptic DA release, which in turn leads to increased post-synaptic D1R activation (Shi et al., 1997). A mixed D1–D2 antagonist with similar affinity for D2R should disinhibit DA release in the same way, but should also lessen the resulting increase in DA signal at post-synaptic D1R. The pattern of effects for pimozide and FLU suggests that a preferential increase in D1R stimulation may not enhance and may even reduce AMPH reinforcement relative to placebo, whereas a balanced reduction in D1R and pre-synaptic D2R signaling may optimize the effects of AMPH on DA transmission and thereby increase its subjective reinforcing properties in healthy volunteers (Brauer and De Wit, 1995).

The present study adopted the dual-antagonist strategy to investigate similarities in the role of D1R in the subjective reinforcing and cognitive effects of gambling activity and AMPH in healthy volunteers. In place of pimozide, we used 3 mg of haloperidol (HAL), the most selective D2R antagonist available for human use in Canada. HAL has similar affinity for D2R (Kᵢ=0.6 nM) as FLU (Kᵢ=0.4 nM) but only 1/20th the affinity (Kᵢ=17.0 nM) for D1R as FLU (Kᵢ=0.85 nM). Because HAL and FLU have similar affinity for other DA receptors (Burstein et al., 2005) and negligible affinity for non-DA receptors (Richelson and Nelson, 1984; Richtand et al., 2007), differences in response to HAL and FLU can be reasonably attributed to differential activation of D1R.

Based on the literature, we predicted that FLU would increase the subjective reinforcing, incentive-motivational, and salience-enhancing effects of the slot machine and AMPH by balancing DA transmission at D1R and D2R, whereas HAL would not enhance and may reduce these effects due to a selective increase in D1R activation. Validated drug effects’ rating scales measured subjective effects, a rapid reading task measured the salience of gambling and emotional words, and blood pressure indexed physiological response to the reinforcers.

Methods and materials

Study design

The study employed a four-session, between-within design. Subjects were matched on age, sex, and trait impulsivity at intake and randomized to the HAL or FLU group (n=15/group). Given that experimental receipt of AMPH has been linked with sensitization in healthy humans (Boileau et al., 2006), AMPH testing was restricted to the final two sessions in order to preclude carry-over effects on responses to the slot machine. Thus, sessions 1 and 2 (Phase I) tested responses to the slot machine, and sessions 3 and 4 (Phase II) tested responses to AMPH. Pretreatment (antagonist, placebo) was counterbalanced within each phase.

Subjects

Healthy volunteers, 19–65 years of age, were recruited via advertisements in print and electronic media. They were prescreened against inclusion/exclusion criteria via telephone. In-depth screening took place on site, where volunteers underwent the Structured Clinical Interview for DSM-IV (SCID-I; First et al., 1995) to rule out Axis I diagnosis (aside from nicotine dependence). To qualify, subjects had to score 0 on the South Oaks Gambling Screen (SOGS; Lesieur and Blume, 1987), have no personal or family history of schizophrenia or bipolar disorder, and have no personal history of substance use disorder. The presence of Axis II psychopathology, conduct disorder, or ADHD was ruled out by an interview with the study psychiatrist. All subjects were drug and medication free based on self-report and urinalysis, and were deemed fit to receive all of the study drugs based on a physician’s exam.

To rule out possible subclinical depressive symptoms, subjects had to score ≤10 on the short form of the Beck Depression Inventory (BDI-sf; Beck and Beck, 1972). To rule out subclinical addiction-related pathology, subjects had to drink no more than 15/12 (male/female) standard drinks/week, score ≤13 (bottom quartile; “low dependence”) on the Alcohol Dependence Scale (Skinner and Horn, 1984), and ≤4 (no drug abuse) on the Drug Abuse Screening Test (DAST; Skinner, 1982). To avoid possible effects of nicotine withdrawal during the four-hour test phase, volunteers who smoked >20 cigarettes per day were ineligible. Given the strong association between smoking and problem gambling (Barnes et al., 2015; Weinberger et al., 2015), this criterion also strengthened the external validity of the present sample with regard to non-problematic gamblers in the community. To minimize novelty effects, all subjects were required to have played a slot machine on at least three previous occasions. Subjects were also required never to have taken a psychostimulant drug (AMPH, cocaine, methamphetamine, or methylphenidate).

Apparatus

Self-report scales. Visual Analogue Scales (VAS: 0–10; Fischman and Foltin, 1991) assessed desire to gamble, and in Phase II, desire to take AMPH again. The Addiction Research Center Inventory (ARCI; Haertzen, 1965) AMP, MBG, and LSD subscales assessed subjective psychostimulant-like, euphoric, and dysphoric effects, respectively. A Side Effects Checklist (Zawertailo et al., 1995), validated for acute psychoactive drug administration studies, assessed possible adverse effects of drugs. Trait scales included: SOGS, the alcohol Timeline Followback (TLFB; Sobell and Sobell, 1992) measure of drinking in the past 90 days, Fagerstrom Test of Nicotine Dependence (FTND; Heatherton et al., 1991), DAST, and BDI-sf. The Eysenck Impulsiveness Scale (EIS; Eysenck et al., 1985) assessed trait impulsivity. The Eysenck Lie scale (Eysenck and Eysenck, 1963) assessed the tendency to respond in a socially desirable manner (i.e., positive image management) to help validate the other self-report data.
Slot machine. A commercial slot machine ("Cash Crop"; WMS Gaming, Detroit, MI) served as the gambling reinforcer. Features of the game were identical to those of our previous studies (Zack and Poulos, 2007, 2009). Subjects received an initial stake of 400 credits ($100) on each session, and could bet 1–45 credits/trial. The game lasted for 15 minutes or until credits ran out, whichever came first. Winning trials (~46%; Tremblay et al., 2011) were accompanied by bells and lights. Trial-by-trial bet size and credits won were recorded electronically. To accomplish this, the cable that sent the video signal to the screen of the slot machine was split and an identical signal was sent to a PC monitor in a closed anteroom. A camcorder recorded all events (credits bet on each trial, payoff on each trial, etc.) that were relayed to the monitor as the subject played the game (the subject was not seen). Each event on the video record was later transcribed manually to the database. To encourage spontaneous betting behavior, subjects were not told that their behavior was being monitored until debriefing, at which time they were allowed to have their betting data excluded from the analysis if they wished (none did).

RRT: cue salience. Task parameters and stimuli on the RRT were identical to our previous studies (Zack and Poulos, 2004, 2007, 2009). Subjects performed 20 practice trials (neutral words only)+150 test trials on each test session with categories and words randomized throughout. Events on each trial were the same: fixation stimulus (&&&&; 350 ms), blank (250 ms), target word (until response), blank (550 ms). They were told to read the same: fixation stimulus (&&&&; 350 ms), blank (250 ms), target word (until response), blank (550 ms). They were told to read the same: fixation stimulus (&&&&; 350 ms), blank (250 ms). Subjects performed 10 practice trials (neutral words only)+150 test trials on each test session with categories and words randomized throughout. Events on each trial were the same: fixation stimulus (&&&&; 350 ms), blank (250 ms), target word (until response), blank (550 ms). They were told to read the same. Each event on the video record was later transcribed manually to the database. To encourage spontaneous betting behavior, subjects were not told that their behavior was being monitored until debriefing, at which time they were allowed to have their betting data excluded from the analysis if they wished (none did).

Data analysis

Chi-square tests of independence compared the sex ratio in the HAL and FLU groups. A multivariate analysis of variance (MANOVA) of trait scale scores, with univariate tests for each scale, compared the groups on all background variables.

A series of 2 (Group)--2 (pretreatment: antagonist, placebo)--2 (phase: slot machine, AMPH)--2 (time: peak antagonist, post-reinforcer) analyses of variance (ANOVAs) assessed self-reported subjective and physiological effects. Difference scores measured the effects of antagonist alone (time 1: post-dose 1 peak minus pre-dose 1 baseline) and antagonist+reinforcer (time 2: post-reinforcer minus post-dose 1 peak). In Phase II, post-reinforcer ratings occurred at 90 minutes post AMPH (peak subject effects of oral AMPH; Brauer et al., 1996).

To compare primed motivation to gamble with primed motivation for AMPH directly, a 2 (group)--2 (pretreatment)--2 (phase) ANOVA analyzed VAS desire to gamble following the slot machine in Phase I and VAS desire to take AMPH again at peak AMPH levels in Phase II. To determine the extent to which the priming effects of gambling and AMPH tapped a common construct, the correlation between post-slot machine desire to gamble and peak AMPH (90 minutes post capsule) desire to take AMPH again was computed for the placebo pretreatment. To assess effects of antagonist pretreatment on this relationship, the correlation between difference scores (antagonist minus placebo) and peak AMPH (90 minutes post capsule) was computed.
was also computed. As difference scores are not appropriate for parametric correlational analysis (Cohen et al., 2003), Spearman’s rank correlation was used for these analyses.

A 2 (group) > 2 (pretreatment) > 2 (phase) > 4 (word category) ANOVA assessed percent neutral word response time (RT; facilitation = test/neutral RT > 100) for gambling, alcohol, and positive and negative words on the RRT. Per standard procedure for vocal RT tasks, individual raw trial RT scores < 200 ms or > 2.5 SD above the mean for the respective word category were designated as outliers and excluded from the analyses. A 2 (group) > 2 (pretreatment) > 3 (measure) MANOVA assessed bet size (credits/spin), speed of play (spins/15 minutes), and winnings (final credit tally) on the slot machine in Phase I.

For all analyses, planned comparisons for hypothesized effects were conducted using the MS and df error terms for the ANOVA (Winer, 1971). Nonparametric analyses, using Wilcoxon’s rank sum test, assessed the difference in the effects of HAL versus FLU relative to placebo, where appropriate.

Results

Subject background characteristics

Table 1 reports the background characteristics for groups HAL and FLU, along with results of the statistical tests. The table shows that the relative frequency of men versus women was somewhat greater in group HAL compared with group FLU, although the disparity was nonsignificant (p > 0.45). Two subjects who enrolled in the study (both in group HAL) dropped out after test session 1 due to akathisia. Their data are not included in the present sample (N = 30).

Only one subject smoked (group HAL), and the pattern of significant effects was the same when this subject was included or excluded from all analyses. Age data indicated that both groups were comprised of mature adults. Group means for the trait scales indicate similar low levels of depression and alcohol and drug use. Both groups spent < 5 drinks/week on average on gambling. Trait impulsivity scores on the EIS were similarly low relative to population norms (μ = 3.9; Eysenck et al., 1985). Scores on the Eysenck Lie Scale exactly matched the population mean in group HAL with a slightly higher (but statistically equivalent) score in group FLU.

These data confirm the similarity of the two groups on background variables and indicate that the sample as a whole was psychologically healthy with low levels of impulsivity and addictive behavior. The Lie score data indicate no tendency to falsify self-report.

Subjective effects

Desire to gamble. A 2 (Group: HAL, FLU) x 2 (Pre-treatment: Placebo) x 2 (Phase: Slot Machine, AMPH) ANOVA of change (Δ) in Desire to Gamble ratings yielded a significant main effect of Time, F (1, 28) = 21.74, p < .001, and no other significant effects. The mean (SE) change in Desire to Gamble was 1.96 (0.21), or Phase I, Δ = 0.02 (.10), or Phase II, Δ = 0.01 (0.12). The Δ in desire due to the slot machine corresponded to an increase of 132% while Δ in desire due to AMPH corresponded to an increase of 111%. Thus, the slot machine and AMPH each increased Desire to Gamble to a statistically equivalent degree, which was more

<table>
<thead>
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<th>Time</th>
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<tbody>
<tr>
<td>1</td>
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<td>11</td>
<td>12</td>
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<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 1. Background characteristics (mean (SD); frequency) for subjects assigned to group HAL (n = 15) or group FLU (n = 15).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group HAL</th>
<th>Group FLU</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>36.1 (11.7)</td>
<td>39.9 (14.2)</td>
<td>0.43</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>1:5</td>
<td>8:7</td>
<td>0.46</td>
</tr>
<tr>
<td>Smokers (Y:N)</td>
<td>1:14</td>
<td>0:15</td>
<td>0.34</td>
</tr>
<tr>
<td>Gambling ($/week)</td>
<td>2.5 (5.8)</td>
<td>3.6 (4.2)</td>
<td>0.55</td>
</tr>
<tr>
<td>Gambling problems (SOGS)</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Alcohol (drinks/week; TLFB)</td>
<td>1.6 (1.6)</td>
<td>1.9 (1.8)</td>
<td>0.64</td>
</tr>
<tr>
<td>Alcohol dependence (ADS)</td>
<td>0.6 (0.9)</td>
<td>0.4 (1.1)</td>
<td>0.58</td>
</tr>
<tr>
<td>Drug abuse (DAST)</td>
<td>0.5 (0.7)</td>
<td>0.4 (0.8)</td>
<td>0.59</td>
</tr>
<tr>
<td>Depression (BDI-sf)</td>
<td>0.9 (1.2)</td>
<td>1.4 (2.1)</td>
<td>0.45</td>
</tr>
<tr>
<td>Impulsiveness (EIS)</td>
<td>2.6 (2.1)</td>
<td>1.6 (2.6)</td>
<td>0.24</td>
</tr>
<tr>
<td>Positive self-report bias (EPI-Lie)</td>
<td>3.9 (2.0)</td>
<td>4.7 (1.9)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

SOGS: South Oaks Gambling Screen (≥ 5 = probable pathological gambling); TLFB: Timeline Followback (drinks per week in last 90 days); ADS: Alcohol Dependence Scale (≥ 9 = alcohol abuse); DAST: Drug Abuse Screening Test (≥ 4 = drug abuse); BDI-sf: Beck Depression Inventory short form (≥ 14 = clinical depression); EIS: Eysenck Impulsiveness Scale (normative μ = 8.5); EPI-Lie: Lie scale of Eysenck Personality Inventory (normative μ = 3.9).
than twice the level before either of these reinforcers and greater than the increase due to either antagonist or placebo alone.

Desire to gamble versus desire to take AMPH again: mean effects. A 2 x 2 x 2 ANOVA directly comparing Desire to Gamble ratings (0-10) immediately after the slot machine with Desire to Take AMPH Again (0-10) at peak AMPH effects yielded a marginally significant Group x Pre-treatment interaction, F (1, 27) = 4.03, p = .055, and no other significant effects. Panels A and B of Figure 2 show the scores for each Group and Phase and reveal that HAL and FLU had differential effects in each Phase. Simple effects analyses found that FLU was associated with significantly higher scores under drug vs. placebo, t (27) = 2.48, p < .05, whereas HAL was associated with significantly lower scores under drug vs. placebo, t (27) = -3.30, p < .01.

In light of the small absolute size and difference in Desire to Gamble scores in Groups HAL and FLU in Phase I, follow-up non-parametric tests compared the difference (Antagonist – Placebo) in Desire to Gamble for the two groups immediately following the slot machine. The mean rank score for HAL, 12.30, was significantly lower than the mean rank for FLU, 18.70, Wilcoxon’s W = 184.50, p = .045 (two-tailed). To confirm that the same pattern was evident in Phase II, the difference (Antagonist – Placebo) in Desire to Take AMPH Again at peak AMPH effects was also compared for each group. The mean rank for HAL, 11.57, was again significantly lower than the mean rank for FLU, 19.43, W = 173.5, p = .013 (two-tailed). The results of these non-parametric tests confirm that, relative to placebo, Desire to Gamble after the slot machine was significantly greater under FLU vs. HAL, and Desire to Take AMPH Again at peak AMPH effects was also significantly greater under FLU vs. HAL.

Desire to gamble versus desire to take AMPH again: correlational effects. Under placebo pretreatment, the correlation between post-slot machine desire to gamble and post-capsule peak AMPH desire to take amphetamine again; (a) under placebo pretreatment in subjects who received HAL (3 mg; n=15) or FLU (3 mg; n=15) on the antagonist test session, and (b) for the effects of pretreatment (antagonist-placebo) in ratings on these two scales in the same subjects.

Figure 2. (a) Mean (SE) desire to gamble (0–10) after the slot machine game in Phase I. (b) Desire to take amphetamine again at peak subjective effects (90 minutes post capsule) of amphetamine (20 mg, oral) in Phase II in healthy volunteers pretreated with haloperidol (HAL, 3 mg; n=15) or fluphenazine (FLU; 3 mg, n=15), and in each antagonist group, under placebo.

Figure 3. Scatterplot and Spearman correlation of rank scores for post-slot machine desire to gamble and for 90 minutes post-capsule peak amphetamine (20 mg) desire to take amphetamine again; (a) under placebo pretreatment in subjects who received HAL (3 mg; n=15) or FLU (3 mg; n=15) on the antagonist test session, and (b) for the effects of pretreatment (antagonist-placebo) in ratings on these two scales in the same subjects.
scores for antagonist–placebo pretreatment for the gambling and AMPH sessions was significant (Spearman \( r_s = 0.364, p = 0.048 \), two-tailed; see Figure 3(b)). Comparison of panels (a) and (b) reveals that the distribution of scores was quite diffuse under placebo, whereas the antagonist effect was associated with increased coherence of scores around the regression line. The plot of difference scores also shows that the correlation was not due solely to a dichotomous distribution of scores under the two antagonists.

**ARCI AMP scale: psychostimulant effects.** A 2×2×2×2 ANOVA of ARCI AMP scale scores yielded main effects of phase (\( F[1, 24] = 9.99, p = 0.004 \)) and time (\( F[1, 24] = 14.34, p = 0.001 \)), along with a phase×time interaction (\( F[1, 24] = 6.46, p = 0.018 \)), a group×pretreatment×time interaction (\( F[1, 24] = 6.20, p = 0.020 \)), and no other significant effects.

Panels (a)–(d) of Figure 4 show the change scores (peak antagonist–baseline; post-reinforcer–peak antagonist) under drug and placebo for each antagonist group. The figure shows that the main effect of time reflected a consistent increase in AMP scores for the slot machine and AMPH dose compared with a lack of change for the antagonist alone. The main effect of phase reflected a relatively greater increase in AMP scores for the AMPH dose versus the slot machine. The three-way interaction arose because, relative to placebo, HAL consistently reduced (\( t[24] = -3.69, p < 0.01 \)) whereas FLU consistently augmented (\( t[24] = 3.01, p = 0.01 \)) the increase in AMP ratings for the slot machine and the dose of AMPH. The lack of four-way interaction indicated that the pattern of effects for HAL and FLU did not differ for the two reinforcers.

**ARCI MBG scale: euphoric effects.** The ANOVA of MBG scores yielded significant main effects of phase, pretreatment, and time (\( F[2, 28] > 4.21, p < 0.05 \)), a phase×time interaction (\( F[1, 28] = 10.82, p = 0.003 \)), and no other significant effects.

Table 2 reports the mean (SE) MBG scale scores as a function of all factors, and indicates that the main effect of phase denoted greater overall scores, collapsed across time points in Phase II versus Phase I; the main effect of time denoted greater overall scores in each phase at time 2 after the reinforcer than time 1 at peak levels of the antagonist; and the interaction denoted a relatively greater increase in MBG scores for AMPH versus the slot machine at time 2. The main effect of pretreatment indicated significantly lower MBG ratings under both antagonists, relative to placebo, both before and after the reinforcers.

**ARCI LSD scale: dysphoric effects.** The ANOVA of LSD scale scores yielded a significant main effect of time (\( F[1, 24] = 7.11, p = 0.013 \)), a marginal group×pretreatment×time interaction
Table 2. Mean change (SE) scores on Addiction Research Center Inventory (ARCI) morphine-benzodrine group (MBG) euphoria scale, LSD dysphoria scale, and diastolic blood pressure at peak antagonist minus baseline (time 1) and post-reinforcer (15-min slot machine game or 20 mg d-amphetamine) minus peak antagonist (time 2) in healthy volunteers (n=15/antagonist group) under haloperidol (HAL; 3 mg), fluphenazine (FLU; 3 mg), or placebo (PLAC).

<table>
<thead>
<tr>
<th>Dependent measure</th>
<th>Phase</th>
<th>Pretreatment</th>
<th>Time 1</th>
<th>Time 2</th>
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<tr>
<td>ARCI-MBG Slots</td>
<td>HAL</td>
<td>−0.13 (0.40)</td>
<td>0.00 (0.35)</td>
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<tr>
<td></td>
<td>PLAC</td>
<td>−0.93 (0.46)</td>
<td>0.73 (0.35)</td>
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<td></td>
<td>PLAC</td>
<td>0.13 (0.46)</td>
<td>−0.53 (0.35)</td>
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<td>AMPH</td>
<td>HAL</td>
<td>−0.20 (0.42)</td>
<td>2.47 (0.99)</td>
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<tr>
<td></td>
<td>PLAC</td>
<td>0.27 (0.42)</td>
<td>3.07 (0.83)</td>
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<tr>
<td></td>
<td>FLU</td>
<td>−1.07 (0.42)</td>
<td>1.47 (0.99)</td>
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</tr>
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<td>PLAC</td>
<td>−0.67 (0.42)</td>
<td>2.33 (0.83)</td>
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<tr>
<td>ARCI-LSD Slots</td>
<td>HAL</td>
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<td>0.29 (0.22)</td>
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<td>−0.14 (0.25)</td>
<td>0.14 (0.26)</td>
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<tr>
<td></td>
<td>FLU</td>
<td>−0.25 (0.30)</td>
<td>0.58 (0.23)</td>
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<td></td>
<td>PLAC</td>
<td>0.08 (0.27)</td>
<td>0.17 (0.29)</td>
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<tr>
<td>AMPH</td>
<td>HAL</td>
<td>0.21 (0.26)</td>
<td>0.07 (0.22)</td>
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<td></td>
<td>PLAC</td>
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<td></td>
<td>FLU</td>
<td>−0.08 (0.28)</td>
<td>1.08 (0.41)</td>
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<tr>
<td>DBP</td>
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<td>−1.20 (1.98)</td>
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<td></td>
<td>PLAC</td>
<td>−3.20 (2.47)</td>
<td>19.13 (6.22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FLU</td>
<td>−4.29 (2.05)</td>
<td>24.93 (5.73)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLAC</td>
<td>−1.29 (2.56)</td>
<td>17.29 (6.44)</td>
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<tr>
<td>AMPH</td>
<td>HAL</td>
<td>−0.60 (1.87)</td>
<td>28.53 (7.62)</td>
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<tr>
<td></td>
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<td>−1.73 (2.10)</td>
<td>26.60 (6.84)</td>
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</tr>
<tr>
<td></td>
<td>FLU</td>
<td>−0.79 (1.94)</td>
<td>30.07 (7.89)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLAC</td>
<td>−4.29 (2.18)</td>
<td>38.07 (7.08)</td>
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</table>

Table 2 reports the mean (SE) DBP scores as a function of all factors, and shows that the main effect of group reflected greater increases in group FLU versus HAL, regardless of pretreatment. The main effect of phase reflected a greater overall increase for AMPH sessions versus gambling sessions. The main effect of time reflected a pronounced increase in DBP from pre to post slot machine and from pre to post AMPH. Thus, both reinforcers significantly increased physiological arousal. The phase×time interaction indicated a ~50% greater increase in DBP after AMPH versus the slot machine. The lack of significant effects involving pretreatment suggests that the group effect was due to stable trait differences in physiological reactivity rather than differential effects of HAL versus FLU.

Physiological effects: diastolic blood pressure

Diastolic blood pressure (DBP) provided a relatively enduring index of physiological response to the reinforcers, as against heart rate and systolic blood pressure (Meyer et al., 2004; Studer and Clark, 2011), which dissipated quickly after the slot machine. The ANOVA of DBP scores yielded significant main effects of group, phase, and time ($F[1, 27]=4.22, p=0.051$), and no other significant effects. Table 2 shows that the main effect of time reflected a reliable increase in LSD scores from pre to post reinforcer in each phase, regardless of pretreatment. The marginal three-way interaction reflected a greater increase in LSD scores from pre to post reinforcer in each phase for FLU versus placebo ($t[24]=3.96, p<0.001$) but not HAL versus placebo ($p>0.10$), and the change in LSD scores from baseline to peak antagonist did not differ significantly for antagonist versus placebo in either group.

Side Effects Checklist. A 2×2×2 ANOVA of scores on the Side Effects Checklist, taken once at the end of each test session, yielded no significant effects. Inspection of the means for each group and session revealed that the rating was somewhat higher for placebo pretreatment plus AMPH (2.85/5 in group FLU), whereas all other mean ratings ranged from 1.2 to 1.9 for each group, phase, and pretreatment. Thus, all combinations of antagonist, slot machine, and AMPH were well tolerated.

RRT: cue salience. The percent neutral response time for each test word category on the RRT assessed the salience of gambling, alcohol, positive affect, and negative affect words immediately after the slot machine and at peak AMPH effects following pretreatment with antagonist or placebo. The 2×2×2×4 (word category) ANOVA yielded significant main effects of group ($F[1, 27]=6.02, p=0.021$), phase ($F[1, 27]=25.53, p<0.001$), and word category ($F[3, 81]=18.08, p<0.001$). Planned comparisons for word category revealed a significant difference for gambling words versus words from the alcohol, positive affect, and negative affect categories ($p<0.019$). In addition, the score for gambling words versus negative words varied significantly as a function of group and pretreatment (simple group×pretreatment×word category interaction; $F[1, 27]=4.57, p=0.042$). This effect is illustrated in panels (a)–(d) of Figure 5, which plot the percent of neutral RT scores (facilitation) in the four test categories under drug and placebo for each group and phase. Smaller scores denote faster relative response time compared with neutral (100%), that is, greater salience.

Inspection of Figure 5 shows that, relative to placebo, HAL increased ($t[27]=2.63, p<0.05$) whereas FLU decreased ($t[27]=−3.06, p<0.01$) the salience of negative affect words (right-most bars in each panel) after both reinforcers. In contrast, neither antagonist reliably decreased and tended to increase the salience of gambling words, although this effect was not significant ($p>0.10$). The directionally opposite effects of HAL versus FLU for negative words, coupled with the generally congruent effects of HAL and FLU for gambling words, accounts for the significant three-way simple interaction for gambling versus negative affect words.

Betting behavior and winnings on slot machine

A 2×2×3 (measure) MANOVA assessed mean credits wagered per spin, total spins per game, and final credit tally for each group under antagonist and placebo in Phase I. Consistent with previous studies, all three variables were positively skewed, and therefore underwent logarithmic transformation prior to analysis.

The MANOVA yielded no significant multivariate or univariate effects. Table 3 reports the untransformed mean (SE) scores for each group and pretreatment, and shows that bet size...
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(maximum 45 credits/spin) was somewhat larger under the antagonist versus placebo in both HAL and FLU groups. Relative to placebo, HAL was associated with somewhat fewer spins (slower play), while FLU was associated with somewhat more spins. Relative to placebo, the antagonist was associated with somewhat greater winnings (randomly determined) in both HAL and FLU groups. Therefore, directional differences in the subjective or cognitive effects of the two antagonists relative to placebo are not attributable to differences in winnings.

**Discussion**

This study investigated the role of D1R in the reinforcing effects of slot-machine gambling and AMPH in healthy volunteers. It was predicted that the preferential D2R antagonist HAL would have opposite effects to the mixed D1–D2 antagonist FLU on subjective reinforcement, incentive motivation, cue salience, and physiological arousal, and that the pattern of antagonist effects would be congruent for the game and AMPH. The results largely supported these hypotheses.
VAS ratings of desire to gamble indicated that both the slot machine and AMPH increased the incentive value of gambling, although the effect was small and not altered by either antagonist. The small increase in desire scores is consistent with the background characteristics of this sample, which indicated very low levels of gambling.

Previous research found that AMPH (17.5 mg/70 kg) changed the emotional response to potential loss in a gambling-like guessing game from negative arousal (e.g., dread) to positive arousal (e.g., hope) in healthy volunteers (Knutson et al., 2004). A similar process may explain the enhanced value of gambling under AMPH in the present study.

Direct comparison of desire to take AMPH again with desire to gamble ratings after the respective reinforcers revealed the effects of the antagonists. FLU enhanced the priming (pro-motivational) effects of the slot machine and AMPH, whereas HAL coincided with a slight decrease in priming effect of the game and marked decrease in priming effect of AMPH. These results indicate that partial D1R blockade (FLU) increases the incentive value of gambling and a moderate dose of AMPH in healthy volunteers. In contrast, unmitigated D1R activation due to preferential D2 autoreceptor blockade (HAL), and consequent disinhibition of DA release, does not enhance the value of gambling and reduces the value of AMPH in this population. Previous research had found that selective stimulation of D1R reduced the incentive value of cocaine in animals familiar with the drug “possibly through satiation of reward pathways” (Self, 1998: 379). By analogy, HAL may have fully satiated motivation for gambling and AMPH, whereas partial blockade of D1R by FLU may have had an appetizing effect for both reinforcers.

The correlational results complemented the analyses of mean effects. Whereas desire to gamble did not significantly correlate with desire to take AMPH again under placebo pretreatment, the effects of treatment (antagonist-placebo) on desire to gamble and to take AMPH again were significantly intercorrelated. The increased coherence in data points about the regression line in the scatterplot of difference scores (Figure 3(b)) suggests that the antagonists revealed the overlap in motivation for gambling and AMPH by amplifying systematic variance in the effects of the respective reinforcers at D1R while reducing unsystematic variance across individuals due to other factors.

The ARC1 AMPH data provided the clearest support for the hypotheses. HAL consistently decreased while FLU consistently increased the psychostimulant-like effects of both reinforcers. Although these results could indicate that D1R activation is unpleasant, the pattern of effects for the antagonists alone at time 1 suggests otherwise. In both phases, FLU alone decreased AMPH-like effects relative to placebo, but increased AMPH-like effects when combined with the game or AMPH. The same within-session bidirectional pattern of AMPH ratings was seen for HAL in Phase I, except in this case, the antagonist alone tended to enhance whereas antagonist+slot machine decreased AMPH ratings. In phase II, HAL alone had no effect but again reduced AMPH ratings when combined with the AMPH dose. Collectively, the within-session and between-group effects suggest that a moderate level of D1R activation coincides with an optimal psychostimulant-like effect in healthy volunteers. These data clearly support the assertion that D1R plays a parallel role in the positive arousing effects of gambling and AMPH in healthy volunteers.

The MBG and LSD scale scores revealed that HAL and FLU also modified subjective euphoria and dysphoria. In this case, the effects largely pertained to AMPH, whereas the slot machine did not yield appreciable effects. Both HAL and FLU decreased the euphoric effects, while FLU also increased the dysphoric effects of AMPH. The finding that both antagonists decreased AMPH-induced euphoria suggests that D2R, which is blocked by both HAL and FLU, may mediate this effect. This aligns with previous research which found that displacement of the D2/3 radioligand, raclopride, in the ventral striatum predicted AMPH-induced euphoria in healthy volunteers (Drevets et al., 2001).

The pattern of effects on the RRT was consistent with the subjective effects of HAL and FLU. The between-group difference emerged in the relative salience of gambling versus negative affective words. Compared with placebo, HAL increased the salience of gambling and negative words after the slot machine, whereas FLU decreased the salience of negative words at this time. In Phase II, HAL decreased the salience of gambling words and increased the salience of negative words, whereas FLU again decreased the salience of negative words. The results for HAL suggest that increased DA release combined with robust stimulation of D1R increased the salience of negative emotional cues (e.g., w*o*r*y) in response to the game and reduced reactivity to gambling cues (e.g., w*a*g*e*r) in response to AMPH. Both results are consistent with a satiating effect of intense D1R activation. The results for FLU suggest that increased DA release coupled with partial D1R blockade did not alter the salience of gambling cues in either phase, but reduced the salience of negative cues in response to both reinforcers, consistent with a possible disinhibiting effect of moderate D1R activation. Because the RRT results are less susceptible to experimental demand, their congruity with the subjective effects supports the validity of the self-report data. However, given that the RRT also included positive affective words, the observed effects of HAL and FLU on response time to gambling versus negative words is only one of several possible results that could be viewed as consistent with the self-report findings.

The DBP data revealed that both the slot machine and AMPH led to significant physiological arousal. This is consistent with previous research from healthy women who gambled in a laboratory setting (Yucha et al., 2007), and with the well-established pressor effects of AMPH. The lack of significant antagonist-related effects on DBP are consistent with previous research showing that the cardiovascular effects of AMPH are largely mediated by norepinephrine (NE) as opposed to DA (Nurnberger et al., 1984). As such, they help to rule out possible downstream effects on NE as an explanation for the subjective and cognitive effects found here.

HAL and FLU had no reliable effects on bet size or speed of play on the slot machine. This is consistent with previous research, which found that the speed of play is important to the reinforcing effects of an electronic gaming device in problem gamblers but not in social gamblers (Loba et al., 2001). With respect to bet size, previous research found that HAL causes a time-dependent change in the relationship between amount won on a given spin and amount wagered on the next spin of a slot machine in healthy volunteers (Tremblay et al., 2011). During early stages of play, HAL strengthens this relationship, but during later stages of play, it decreases it—a pattern not unlike an extinction burst followed by extinction in operant responding. It
is possible that this bidirectional pattern contributed to the lack of effects of HAL (and FLU) on overall mean bet size found here. A full trial-by-trial analysis of betting patterns is beyond the scope of the present study, but is an important matter for future investigation to clarify the role of D1R in patterns of overt betting behavior in healthy individuals.

With regard to subjective reinforcement, the present study used several scales (e.g., ARCI MBG, LSD scales) that could conceivably have supported the hypotheses. Although use of multiple non-independent outcome measures is common in studies testing effects of an acute drug challenge (Carter and Griffiths, 2009), including the original studies on AMPH and FLU (Brauer and De Wit, 1995), this approach can increase the chances of a type I error. The consistency of effects across multiple indices in the present study, although not eliminating this concern, does mitigate it to some degree.

The finding that D1R mediates the psychostimulant-like, incentive-motivational, and salience-enhancing effects of gambling in healthy volunteers is important, as it indicates a direct link between gambling and a biological substrate specifically implicated in AMPH sensitization. As such, it supports the possibility that neuroplasticity akin to that induced by chronic AMPH exposure may contribute to the development of PG in individuals chronically exposed to gambling. The emergence of these effects in the absence of gambling pathology indicates that gambling itself can be an addictive agent in a manner directly analogous to drugs of abuse and particularly AMPH. Ultimately, chronic exposure to gambling may be necessary to induce PG. However, individuals who experience the acute effects of gambling as subjectively positive and desirable are more likely to seek out this activity. The present data suggest that individuals with low baseline D1R signaling may be at increased risk for PG relative to individuals with normal or high baseline D1R signaling.

The apparent dissociation between the psychostimulant, euphoric and dysphoric effects of gambling, and AMPH with respect to D1R is also noteworthy, as it may shed light on the distinct features of gambling that contribute to its “psychostimulant-like” profile. Although D1R contributes to numerous psychostimulant effects, perhaps its most pivotal role is in the establishment of reward—the process by which a cue or context becomes associated with a positive or survival-enhancing experience (Beninger et al., 1989). Given that gambling confers its effects entirely by environmental signals, it makes sense that a process that is central to the conditioned reinforcing properties of drugs should play a central role in the positive subjective and incentive motivational properties of this activity. Indeed, in rats, micro-injection of AMPH into the ventral striatum elicits vocalizations that coincide with “reward anticipation” (Burgdorf et al., 2000, 2001) and phasic DA release (Scardochio et al., 2015), which preferentially activates D1R (Schultz, 1998). Thus, the parallel role of D1R in gambling and AMPH reinforcement may primarily apply to the appetitive effects of both reinforcers.

Along with its effects on DA, AMPH also promotes endogenous opioid release in multiple brain regions in healthy volunteers (Mick et al., 2014). Relative to controls, subjects with PG show similar availability of μ opioid receptors but decreased AMPH-induced (0.5 mg/kg) opioid release, and associated reductions in euphoria and alertness under AMPH (Mick et al., 2016). These results contrast with the increase in AMPH-induced (0.4 mg/kg) DA release and increased motivation to gamble (but similar pleasurable effects as controls) found in our previous study of PG subjects (Boileau et al., 2014). The DA and opioid systems interact extensively in the healthy brain, and alterations in their interaction have been implicated in addiction-related neuropathology (Picetti et al., 2013). The opioid antagonist naltrexone reduces the subjective reinforcing effects of acute AMPH in healthy volunteers as well as AMPH-dependent individuals (Jayaram-Lindstrom et al., 2004, 2008). Chronic naltrexone has also shown some clinical benefits in PG patients (Grant et al., 2008). In the ventral striatum, μ receptors register the experience of reward (Laurent et al., 2014), while in the dorsal striatum, μ receptors inhibit D1R-mediated stimulation of adenylyl cyclase (Noble and Cox, 1995). Conversely, D1R blockade negates morphine-induced enhancement of place preference induced by local infusion of a μ agonist (DAMGO) in the ventral tegmental area (Naria et al., 2010), while in the ventral pallidum D1R activation is permissive to the motor stimulant effects of DAMGO (Alesdatter and Kalivas, 1993). Collectively, these findings suggest that a disturbance in complex feedback mechanisms mediated by D1R and μ opioid receptors may contribute to excessive reward seeking in individuals with PG.

The results of the present study suggest that drugs that influence D1R signaling may have therapeutic potential for PG. In line with this, the selective D1R antagonist SCH23390 (ecopipam) has been found to help reduce cravings in an open-label study with PG patients (Grant et al., 2014). The present findings for FLU suggest that although partial D1R blockade (i.e., at sub-saturation doses) may be beneficial in the absence of gambling, this effect may actually enhance the rewarding effects of gambling if a person were to gamble while taking a D1R antagonist. An alternative strategy therefore may be to employ a D1R agonist to “satisfie” the appetite for gambling, in line with the effects of D1R agonists on AMPH and cocaine reward (Hunt et al., 1994; Self, 1998). Consistent with this possibility, the NMDA-antagonist memantine, which increases D1R and D2R signaling, has also proven beneficial for PG in an open-label study (Grant et al., 2010). Ultimately, baseline D1R sensitivity may influence whether an agonist or antagonist strategy is preferred for a given individual with PG. Combination therapy with an indirect D1R agonist and μ opioid antagonist has also been proposed for treating stimulant dependence (Zhu et al., 2011). Given the benefits of μ opioid antagonists for PG, and the parallel role of D1R in gambling and AMPH reinforcement found here, such a combined strategy could provide a broader range of therapeutic effects and greater flexibility in customizing doses to the specific needs of different individuals with PG.

Future clinical considerations must be tempered by the experimental nature of the present data. First, whether HAL and FLU would have similar effects in PG subjects remains to be determined. Second, because the present study used a dual-agonist approach to isolate D1R effects, the impact of selective D1R blockade in the absence of pre-synaptic D2R blockade (cf. Usiello et al., 2000) cannot be established conclusively from these data. Third, the apparent non-linear relationship between D1R activation and subjective-behavioral effects suggests that factors that influence D1R availability such as age, sex, and personality (which all influence the etiology of PG), could impact response to a D1R ligand. Similarly, different doses of D1R medications may have directionally opposite effects in a given individual.
The present findings clarify the function of D1R in the reinforcing effects of gambling, and indicate a parallel role in the acute reinforcing effects of AMPH in healthy individuals. The critical contribution of D1R to conditioned aspects of addictive behavior may help to explain its prominent role in gambling, where environmental signals are the exclusive currency of reinforcement.

Declaration of conflicting interests
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Notes
1. In the present study, the term “priming” is used to describe the effects of an environmental stimulus (slot machine or AMPH) on relative response time to clinically relevant versus neutral word stimuli. Whereas conventional semantic priming tasks utilize primes (usually words) that are presented immediately before each target word (to which the subject makes a vocal reading response), the priming stimuli in the present case were extrinsic to the task. In this sense, the term priming is more compatible with the procedure used in animal studies, where the effects of an experimenter-administered dose of a drug on motivation for that drug—as revealed by subsequent operant drug-self-administration—or for a related drug (cross-priming) are compared to motivation for the drug following an experimenter-administered dose of placebo. In the present design, the reading task was always administered after the active prime stimulus (slot machine/AMPH), and “priming” is intended to denote the preferential activation of clinically relevant concepts and corresponding facilitation in response time to clinically relevant versus neutral words.
2. It is legitimate to perform simple-effects analyses in the absence of a significant overall F score in ANOVA when an a priori hypothesis exists (Howell, 1992).

References


