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ORIGINAL INVESTIGATION

Amphetamine effects on startle gating in normal women and female rats

Jo A. Talledo · Ashley N. Sutherland Owens · Tijmen Schortinghuis · Neal R. Swerdlow

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Abstract
Background Dopamine agonists disrupt prepulse inhibition (PPI) of startle in male rodents. In humans, this is observed only in some studies. We reported that PPI was disrupted by D-amphetamine in men, but only among those with high basal PPI levels. Here, amphetamine effects on PPI were tested in normal women and female rats.
Materials and methods Acoustic startle and PPI were tested in normal women after placebo or 20 mg amphetamine, in a double-blind, crossover design, and in female rats after vehicle or 4.5 mg/kg amphetamine. Rats were from Sprague–Dawley (SD) and Long Evans (LE) strains that differ significantly in gene expression in PPI-regulatory circuitry, including levels of nucleus accumbens (NAC) catechol-*O*-methyl transferase (COMT) mRNA.
Results Amphetamine was bioactive in humans based on quantitative autonomic and self-rating measures, but did not significantly change startle magnitude or PPI across all subjects. Amphetamine's effects on PPI in women correlated significantly ($p<0.0008$) with placebo PPI levels (reducing PPI only in women whose basal PPI levels exceeded the sample median) and with measures of novelty and sensation seeking. Amphetamine decreased PPI in SD rats that have relatively low NAC COMT gene expression and increased PPI in LE rats that have relatively high NAC COMT gene expression.
Conclusion The dopaminergic regulation of PPI in humans is related to basal levels of sensorimotor gating and to specific personality traits in normal men and women. In rats, the effects of amphetamine on PPI differ significantly in strains with low vs. high NAC COMT expression.

Keywords Amphetamine · Dopamine · Prepulse inhibition · Sensorimotor gating · Startle · Strains

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Introduction

Prepulse inhibition (PPI) is an operational measure of sensorimotor gating, in which a startle response to an intense stimulus is automatically suppressed by a weak lead stimulus (Graham 1975). In humans, PPI is typically assessed using electromyographic measures of the blink response, while in rodents, PPI is typically assessed based on quantification of whole body startle. PPI deficits are found in several neuropsychiatric disorders (Braff et al. 1978; cf. Braff et al. 2001), and the biology of these deficits has been the focus of intense study in humans and animal models. Findings related to the neural regulation of PPI suggest both similarities and differences across species. For example, while the indirect DA agonist amphetamine (AMPH) generally disrupts PPI in male rodents via its dopamine-releasing effects in the nucleus accumbens (NAC; Swerdlow et al. 1990; Zhang et al. 2000), it was reported to reduce PPI only in specific subgroups of humans, distinguished by smoking history or personality features (Kumari et al. 1998; Hutchison and Swift 1999).

We failed to detect significant PPI-disruptive effects of 20 mg AMPH in normal men, using stimuli limited to a 100-ms prepulse interval, but subsequently detected PPI-disruptive effects of this same dose of AMPH in normal men, using a paradigm that included very short (10–20 ms) prepulse intervals (Swerdlow et al. 2003b). However, even in this sample, it was determined that the PPI-disruptive

effects of AMPH largely reflected its impact on men with the highest basal levels of PPI. More recently, Bitsios et al. (2005) reported similar findings with other DA agonists: both pergolide and amantadine reduced PPI among men with basal PPI levels above the median of the normal distribution, and actually increased PPI among men with basal levels below the median of this distribution. Thus, the emerging model for the DAergic regulation of PPI in humans is that it differs across individuals in a rate-dependent fashion. If this were simply a matter of PPI "range" or a "regression to the mean", then long prepulse intervals (which evoke higher levels of PPI) would be more sensitive to AMPH, compared to short prepulse intervals (which evoke lower levels of PPI); if anything, the opposite appears to be the case (Swerdlow et al. 2003b).

Normal personality traits, and presumably their underlying neurobiology, also appear to be associated with differences in PPI drug sensitivity. Both the ability of AMPH to reduce PPI, and the ability of antipsychotics to increase PPI, appear to be enhanced among normal individuals with high scores on temperament measures of novelty seeking (Hutchison et al. 1999; Swerdlow et al. 2006). While it might seem far-fetched to propose a biological relationship between the neurochemical regulation of reflex modification and a personality dimension, it has long been known that psychophysiological measures ranging from auditory and visual evoked potentials to PPI are consistently and strongly linked to personality subtypes (e.g., Hegerl et al. 1989; Zuckerman 1990; Stenberg et al. 1988; Lukas 1987; Juckel et al. 1995; Swerdlow et al. 1995). Perhaps most importantly, high levels of novelty seeking in women are associated with specific DA-linked genetic polymorphisms, including the Met/Met genotype of the COMT Val158Met polymorphism, conveying low activity to the enzyme catechol-*O*-methyl transferase (Golimbet et al. 2007). This Met/Met genotype yields COMT with significantly lower activity than its Val/Val counterpart, which might lead to a slower degradation of dopamine after presynaptic release. In previous studies with male rats, we reported that the PPI-disruptive effects of AMPH were significantly greater in Sprague–Dawley (SD) rats than in Long Evans (LE) rats (Swerdlow et al. 2003c), and that COMT gene expression in the NAC is significantly lower ($p<10^{-17}$) in SD vs. LE rats (Shilling et al. 2008). Conceivably, low levels of regional COMT gene expression might result in low levels of enzymatic activity, analogous to the low activity associated with the Met/Met allele most prominent among high novelty seeking individuals.

All of the data reporting "rate-dependent" DA agonist effects on PPI come from studies in men, and the present study was designed to examine this issue in women. PPI is sexually dimorphic: levels of uninstructed PPI (i.e., without attention directed to the prepulse or pulse) are generally higher in normal men compared to normal women (Swerdlow et al. 1993). PPI levels in women also appear to be sensitive to hormonal regulation, as they shift across the menstrual cycle (Swerdlow et al. 1997; Jovanovic et al. 2004) and during pregnancy (Kask et al. 2008). Given the apparent sex differences in the neural control of PPI, it would not be reasonable to assume that patterns of PPI AMPH sensitivity in women would reproduce those in men. We now report the effects of AMPH (20 mg) on PPI in normal women, in relationship to both baseline (placebo) levels of PPI, and normal personality dimensions. These findings were then extended to female rats from strains that differ significantly in levels of COMT gene expression in the NAC.

Materials and methods

Human testing

The methods used in these studies were very similar to those used in studies described in recent reports (Swerdlow et al. 2003b), were approved by the UCSD Human Subjects Institutional Review Board, and were approved and supported by the National Institute of Mental Health. Twenty R handed women (Table 1) completed testing; the study involved phone contact and three laboratory visits. Phone screening procedures were identical to those described in previous reports from our group (Swerdlow et al. 2002, 2003b).

After passing a telephone interview, subjects came to the laboratory within 72 h of menses onset. During a screening examination, the senior investigator (NRS) informed subjects of the potential risks and benefits of the study. Subjects read and signed a consent for study participation, underwent a physical examination and electrocardiogram to rule out exclusionary medical conditions, and completed a urine toxicology test with exclusion for any drug, and a

Table 1 Subject characteristics

Age (mean (range), years)	25.2 (18–32)
Weight (mean (range), kg)	61.7 (47.6–90.3)
Dose AMPH (mean (range), mg/kg)	0.32 (0.22–0.42)
Daily caffeine intake (mean (range), mg)	126.1 (0.0–768.0)
Personality scale scores (mean (range))	
TPQ	
Novelty seeking	15 (8–23)
Harm avoidance	9 (0–18)
Reward dependence	20 (9–27)
SSS	
Total score	18 (7–30)
EPQ	
Total score	20 (15–26)

pregnancy test. Audiometry confirmed hearing threshold <40 dB(A) at 1,000 Hz. Subjects also completed a limited test of the acoustic startle reflex to screen for a minimum eyeblink startle magnitude of 50 units (1.22 μV/unit) using 118 dB(A), 40 ms noise pulses; seven subjects were excluded for failing to meet this criteria.

Subjects completed the following questionnaires: (1) the Tridimensional Personality Questionnaire (TPQ; Cloninger 1987) to assess the relationship between novelty seeking scores (NS) and sensitivity to the effects of AMPH on PPI, based on reports that high NS individuals are most sensitive to the PPI-disruptive effects of AMPH (Hutchison et al. 1999) and the PPI-enhancing effects of antipsychotics (Swerdlow et al. 2006); (2) the Sensation Seeking Scale (Zuckerman et al. 1972), based on reported increased sensitivity to AMPH in individuals scoring high on this measure (Hutchison et al. 1999); and (3) the Eysenck Personality Questionnaire (EPQ; Eysenck and Eysenck 1994). Subjects who passed screening criteria were tested 6–8 days later, and retested 28–30 days after their first experimental session, at the corresponding date in their next menstrual cycle (i.e., approximately the same number of days from menses onset). This schedule was designed to ensure, to the degree possible, that PPI testing with AMPH and placebo occurred under relatively comparable hormonal states—and thus was not confounded or made more variable by hormonal differences—and this was confirmed by measurements of plasma estradiol on both testing days (see below). Testing was double-blind, and drug order was randomized.

On test days, subjects arrived at 0830, ate a standardized breakfast, had a venopuncture for estradiol levels, and D-amphetamine (20 mg) or placebo was administered at 0930. Startle testing began 60 min after pill administration. Heart rate and blood pressure were determined (sitting position, brachial cuff), and subjects completed a symptom rating scale every 30–45 min, the first one before pill ingestion. Symptom rating visual analog scales (VAS) were designed to assess general somatic and psychological symptoms and level of consciousness (modified from Norris 1971; Bond and Lader 1974; Bunney et al. 1999). Subjects made a single, vertical mark representing their current state along on a 100-mm line (0 mm represents "not true" and 100 mm represents "true"). Ratings assessed several states: "happy", "queasy", "dizzy", "drowsy", and perceptual sensitivity. Details of these rating scales are found in Swerdlow et al. (2002) and included prompts such as "Normal sounds seem unusually intense or loud".

For startle testing, subjects sat upright and were directed to look straight ahead, and to stay awake. Two miniature Ag/AgCl electrodes were positioned below and to the outer canthus of each eye over orbicularis oculi; ground electrode was positioned behind the L ear (R<10 kΩ). EMG activity was band-pass-filtered (1–1,000 Hz) and 60 Hz notch-filtered, digitized, and 250 1 ms readings were recorded starting at startle stimulus onset. Acoustic startle stimuli were delivered by Telephonics (TDH-39-P, Maico) headphones. A background 70 dB(A) white noise was continuous throughout the session. Test sessions began with a 3-min acclimation period; during this period, the number of spontaneous eyeblinks were counted by a remote observer using a RadioShack security camera system (model 49-2513; inter-observer R=0.97). This was followed by 42 trials with six conditions repeated in pseudorandom order: a 118-dB(A) 40 ms noise burst alone (pulse alone), and the same 118 dB (A) 40 ms noise burst preceded 10, 20, 30, 60, or 120 ms by a prepulse (5 ms burst) 16 dB over background. A variable inter-trial interval averaged 20 s (15–25 s). The test session was structured identically to that described in our previous studies of AMPH effects on PPI in men (Swerdlow et al. 2003b). On completion of this startle test, additional autonomic and subjective rating measurements were obtained, as were additional "pilot" psychophysiological measures, including a visual latent inhibition task. Data from these subsequent tests are not included in this analysis.

The primary reasons for disqualification were that subjects had low screening startle magnitude (n=7), withdrew from testing prior to the second test day (n=6) or SCID-based diagnosis (n=6); others included positive urine toxicology (n=4) and medical exclusion (n=2).

PPI was defined as (100 − [100 × magnitude on prepulse trial/magnitude on pulse alone trial]). Baseline PPI was normally distributed (mean (SD) % across all intervals= 9.46 (20.32); median=14.18; skewness=−0.58; kurtosis= −0.05). Startle magnitude and PPI were analyzed with mixed-design ANOVAs, with trial type and drug dose as within-subject factors. No consistent drug interactions were noted with eye side (left vs. right), and thus the main effects of eye side and interactions are not reported. VAS ratings were treated as continuous variables and were analyzed with mixed-design ANOVAs. Specific post hoc comparisons were made with one-factor ANOVAs or the Fisher's protected least significant difference test. Alpha was 0.05. In most cases, post hoc comparisons were limited to tests of specific a priori hypotheses (e.g., that DA agonist effects on PPI would be rate-dependent) or planned comparisons (e.g., relationship of PPI AMPH sensitivity to personality measures or estradiol levels).

Rodent testing

The methods used in this study were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by the UCSD Animal Subjects Committee (protocol #S01221). Adult female SD (n=10) and LE rats

(225–250 g; Harlan Laboratories, San Diego, CA, USA) were housed in groups of two to three animals per cage and maintained on a reversed light/dark schedule with water and food available ad libitum. Rats were handled within 2 days of arrival. Testing occurred during the dark phase. D-amphetamine sulfate (AMPH) was dissolved in saline vehicle and administered subcutaneously in doses of 0 or 4.5 mg/kg (as in Swerdlow et al. 2003c). Startle chambers (San Diego Instruments, San Diego, CA, USA) were housed in a sound-attenuated room and consisted of a Plexiglas cylinder 8.2 cm in diameter resting on a 12.5× 25.5-cm Plexiglas frame within a ventilated enclosure. Noise bursts were presented via a speaker mounted 24 cm above the cylinder. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced motion from within the cylinder. Stimulus delivery was controlled by the SR-LAB microcomputer and interface assembly, which also digitized (0-4095), rectified, and recorded stabilimeter readings. One hundred 1-ms readings were collected beginning at stimulus onset. Startle amplitude was defined as the average of the 100 readings.

Approximately 7 days after shipment arrival, rats were exposed to a short "matching" startle session. They were placed in the startle chambers for a 5-min acclimation period with a 70-dB(A) background noise, and then exposed to a total of 17 P-ALONE trails (40 ms–120 dB (A) noise bursts) that were interspersed with 3 PP12dB + P-ALONE trials (P-ALONE preceded 100 ms (onset-to-onset) by a 20-ms noise burst of 12 dB above background). Rats were assigned to dose order groups based on average %PPI from the matching session to ensure similar baseline PPI levels between groups. Four days later, rats were injected with AMPH (0 or 4.5 mg/kg), and 10 min later placed in the startle chambers for a 5-min acclimation period with a 70-dB(A) background noise. They were then exposed to a series of trial types identical to those used in testing humans (see above, "Human testing"). One week later, testing was repeated, with AMPH dose reversed. Statistical analyses of startle magnitude and PPI were structured identically to those used in humans, except that strain was a between-subject grouping factor.

Results

Human testing

Three subjects were startle "non-responders" during testing; two of these subjects were "non-responders" for both placebo and AMPH weeks. Among the 17 remaining subjects, autonomic and VAS measures provided evidence that this dose of AMPH was "bioactive" at the time of PPI testing: AMPH (20 mg) significantly increased resting heart rate (F=5.39, df 1, 16, p<0.035), diastolic and systolic blood pressure (F's=11.23 and 8.99; p's<0.005 and 0.009, respectively), and reduced drowsiness (F=9.25, df 1, 16,

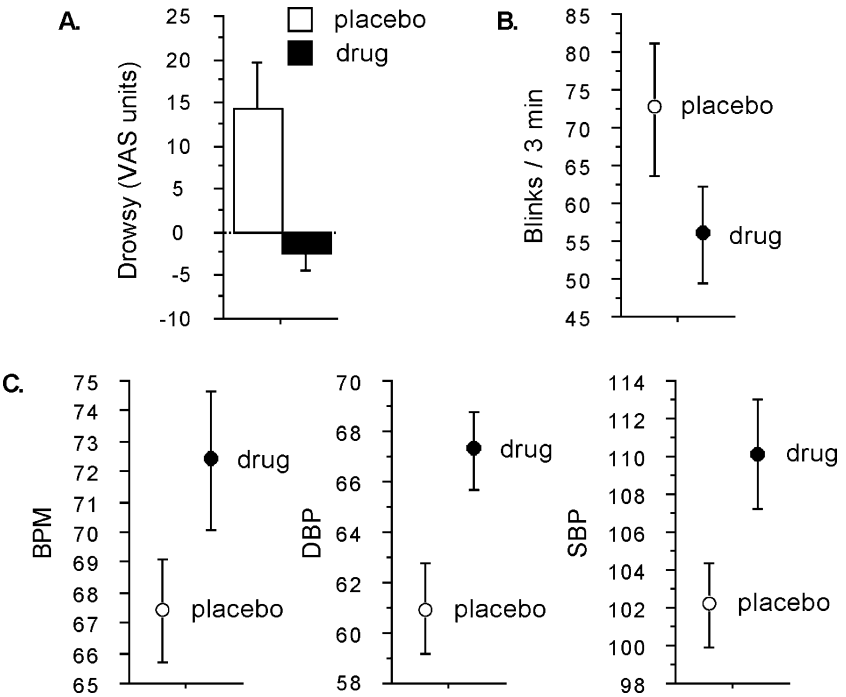

Fig. 1 Evidence of bioactivity of 20 mg AMPH in this study. **a** AMPH prevented the drowsiness normally experienced by test subjects. **b** AMPH also reduced blink rate, an effect likely linked to a reduction in drowsiness. **c** AMPH also increased heart rate (*BPM* beats per minute), and both diastolic and systolic blood pressure (*DBP* and *SBPP*, respectively, in mm)

$p<0.008$) and blink rate ($F=5.83$, df 1, 16, $p<0.03$; Fig. 1). In contrast, this dose of AMPH had no significant effect on perceived queasiness, dizziness, or sensory sensitivity (all were arithmetically reduced by AMPH, but p's were >0.05).

There was no significant main effect of AMPH on PPI ($F<1$), or significant interactions of AMPH dose × prepulse interval ($F<1$). As planned, subjects were then divided based on the lowest vs. highest 50% baseline PPI levels ("low gaters" vs. "high gaters"). ANOVA revealed a significant interaction between AMPH dose and baseline PPI level ($F=8.23$, df 1, 15, $p<0.012$; Fig. 2). This was true: (1) when the "orphan" median value was assigned to either "low" or "high" groups, (2) when only extreme terciles ($ns=5$ each) of the distribution were included, (3) when "non-responders" were included or excluded from analyses, and (4) when active pill order was included as a grouping factor. Among "high gaters", AMPH significantly reduced PPI ($F=17.75$, df 1, 8, $p<0.003$); among "low gaters", PPI-enhancing effects did not reach significance ($F=2.17$, df 1, 7, ns; $d=0.35$, 0.69, 0.79, 0.55, and 0.32 for 10, 20, 30, 60, and 120 ms prepulse intervals, respectively). Regression analysis across all subjects revealed a highly significant correlation between higher baseline PPI level and greater PPI-reducing effects of AMPH ($R=0.74$, $p<0.0008$).

Startle exhibited normal reflex habituation across the test session ($p<0.0001$). AMPH had no significant effect on startle magnitude ($F=3.09$, df 1, 16, ns; Fig. 2, inset) or reflex habituation ($F<1$) across the test session. There were also no differences in startle magnitude or habituation across "low- vs. high-gating" subgroups or subgroup × AMPH interactions for these measures, and all statistical outcomes, including correlations, remained unchanged when the sample was paired so that the arithmetic impact of AMPH on startle magnitude was ≪ 1% for both low- and high-gating subgroups. Regression analyses revealed no significant correlations between: (1) baseline PPI and startle magnitude; (2) baseline PPI and the "AMPH effect" on startle magnitude (mean startle after AMPH minus mean startle after placebo), or (3) the "AMPH effect" on startle and the "AMPH effect" on PPI (all $p>0.05$).

Plasma estradiol levels did not differ significantly between AMPH test days and placebo test days ($F<1$), were reliable (test 1 vs. test 2, $R=0.55$, $p<0.025$), and did not correlate significantly with either baseline PPI levels ($R=0.04$) or AMPH effects on PPI ($R=0.23$).

Analyses of personality correlates of physiological measures were initially limited to test specific relationships of AMPH effects on PPI to both novelty seeking (NS subscale score) and sensation seeking (SSS total score), based on previously published findings (Hutchison et al. 1999). It should be noted that these two scores were highly correlated here ($R=0.75$, $p<0.0007$), as reported elsewhere (Earleywine et al. 1992; Juckel et al. 1995). AMPH effects on PPI correlated significantly with NS scores (higher NS score associated with greater PPI-reducing effects of AMPH, $R=0.54$, $p<0.027$) and SSS scores (higher total SSS score associated with greater PPI-reducing effects of AMPH, $R=0.64$, $p<0.006$), but did not correlate with other TPQ scores or the EPQ Psychoticism or Extraversion subscales. ANOVAs of PPI using median splits of these scales revealed a significant interaction of AMPH × NS ($F=5.18$, df 1, 17, $p<0.04$), and a similar trend for AMPH × SSS ($F=3.29$, df 1, 15, $p<0.09$); in each case, subjects in the upper 50% of the scale exhibited PPI-reducing effects of AMPH (F's=21.38 and 6.69; p's<0.003 and 0.04, respectively), while those in the lower 50% tended to exhibit PPI-enhancing effects of AMPH (Fig. 3). ANCOVAs of the "AMPH effect" (mean PPI after AMPH minus mean PPI after placebo) using baseline PPI as the main factor and NS or SSS scores as covariates in each case revealed significant effects of baseline PPI ($p<0.03$ and $p<0.02$, respectively) and personality scale ($p<0.03$ and $p<0.02$, respectively), and no significant interaction of

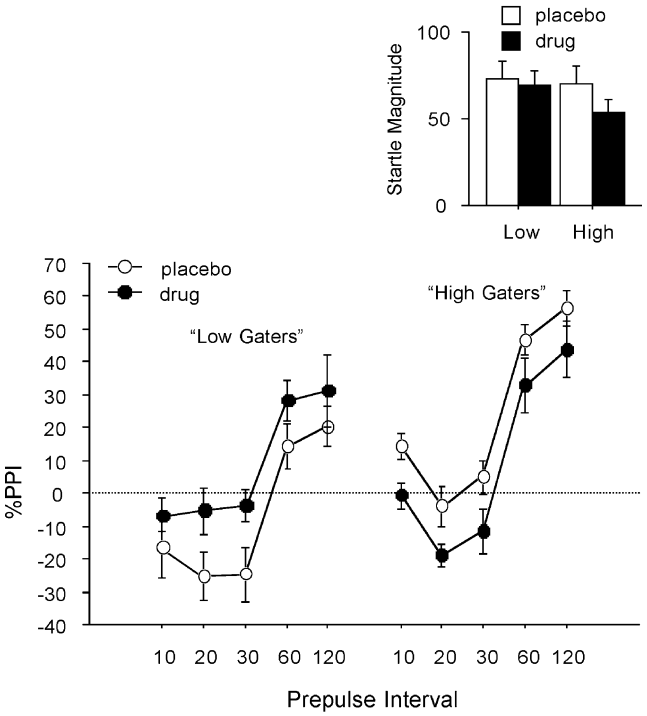

Fig. 2 Effects of AMPH ("drug") vs. placebo on PPI among subgroups defined by median split of mean placebo PPI level. AMPH significantly reduced PPI among "high-gating" subjects ($p<0.003$); in "low-gating" subjects, PPI-enhancing effects of AMPH did not reach significance, despite effect sizes between 0.55 and 0.79 for 20, 30, and 60 ms prepulse intervals. Regression analysis across all subjects revealed a highly significant correlation between higher baseline PPI level and greater PPI-reducing effects of AMPH ($R=0.74$, $p<0.0008$). *Inset* shows no significant effect of AMPH on startle magnitude in low- or high-gating subgroups

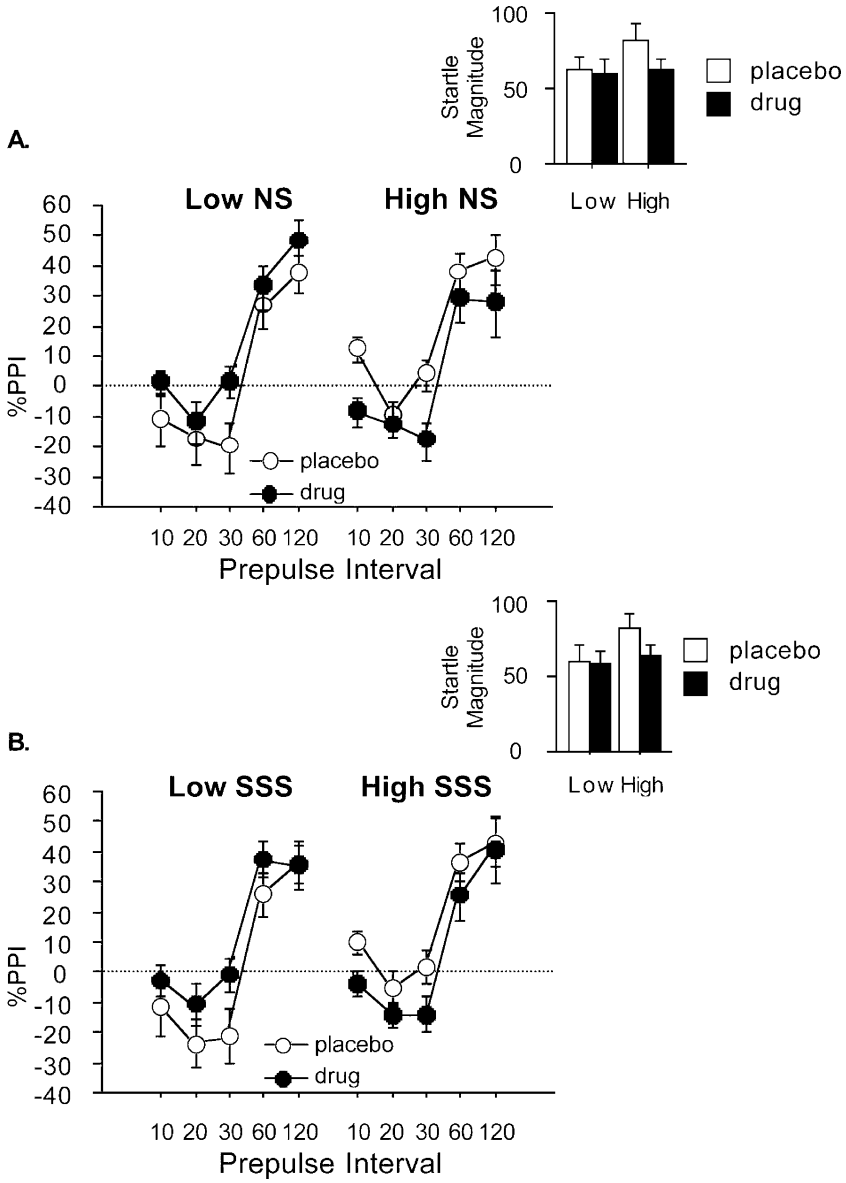

Fig. 3 AMPH effects on PPI among subgroups based on median split of personality scale scores. **a** ANOVA revealed significant PPI-reducing effects of AMPH in subgroups characterized by high NS scores ($p<0.003$). AMPH effects on PPI correlated significantly with NS scores (higher NS score associated with greater PPI-reducing effects of AMPH, $R=0.54$, $p<0.027$. *Inset* shows no significant effect of AMPH on startle magnitude in low or high NS subgroups. **b** Statistically comparable findings were detected using median split analyses for SSS scores (significant PPI-reducing effects of AMPH in the subgroup characterized by high SSS scores ($p<0.04$), and AMPH effects on PPI correlated significantly with SSS scores (higher total SSS scores associated with greater PPI-reducing effects of AMPH ($R=0.64$, $p<0.006$)), and no significant effect of AMPH on startle magnitude in low or high SSS subgroups)

Table 2 Personality scale scores vs. AMPH sensitivity

Scale	Physiological effect of AMPH	*R*	*p*
NS	Reduce PPI	0.54	<0.027[a]
NS	Increase DBP	0.52	<0.035
NS	Reduce blink rate	0.52	<0.035
RD	Increase HR	0.63	<0.008
RD	Increase "happy" VAS	0.56	<0.02
SSS	Reduce PPI	0.64	<0.006[a]

[a] Confirms specific a priori hypotheses based on published reports

baseline PPI and personality scale (both ns). Neither NS nor SSS scores correlated significantly with basal PPI levels (as previously reported (Swerdlow et al. 2003c)), and "low gaters" and "high gaters" did not differ significantly in either NS scores or SSS scores (F's=2.36 and 2.05, respectively, both ns). In contrast, baseline PPI levels were positively associated with reward dependence ($R=0.51$, $p<0.04$) and tended to be associated with Harm Avoidance ($R=-0.48$, $p=0.052$) subscales of the TPQ. Based on these findings, we explored the relationships

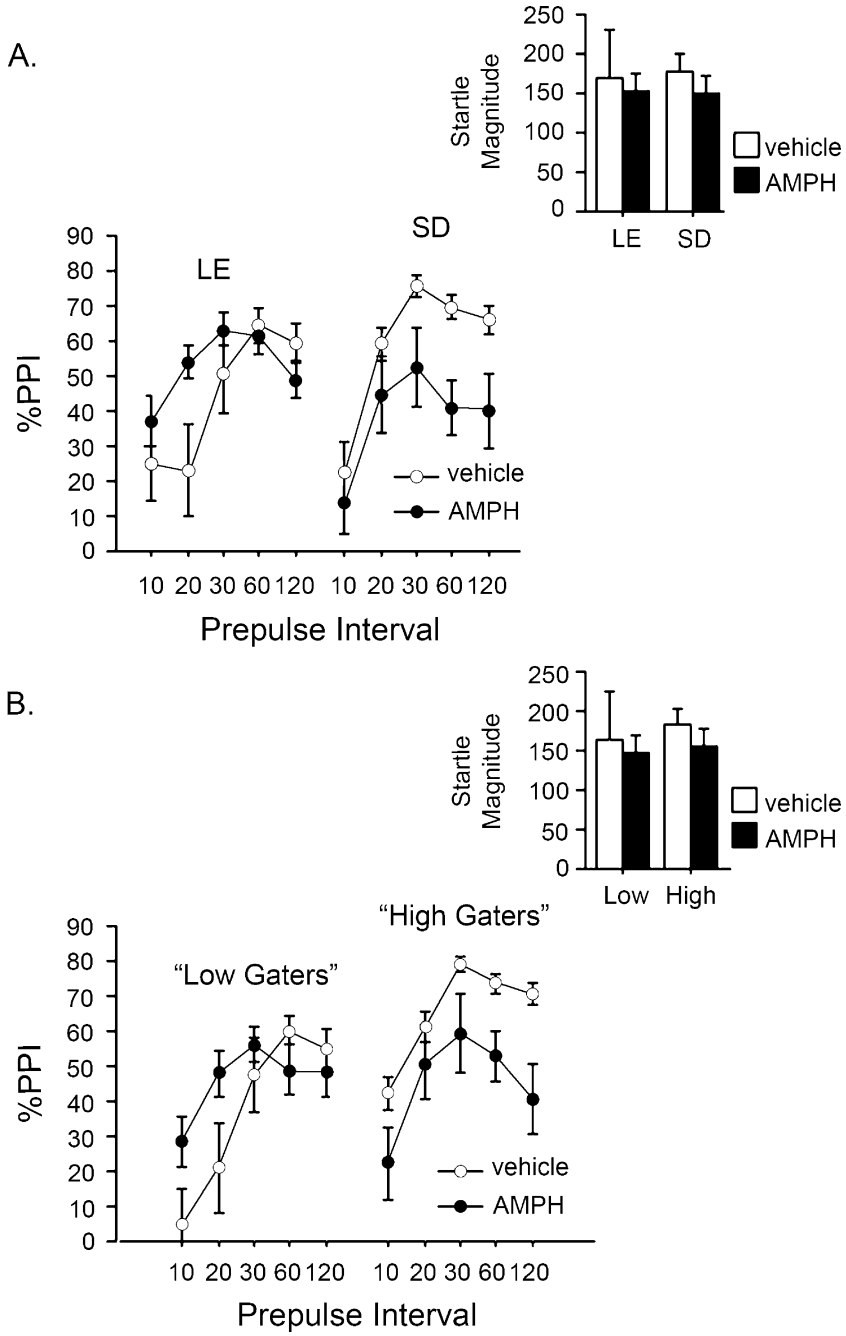

Fig. 4 AMPH effects on PPI in female SD and LE rats, divided by strain (**a**) and low vs. high baseline PPI levels (**b**). SD and LE rats differ significantly in the expression of a number of DA-linked genes in the nucleus accumbens; for example, expression of COMT mRNA is significantly lower in SD vs. LE rats ($p<10^{-17}$). **a** ANOVA revealed significant PPI-reducing effects of AMPH in SD rats ($p<0.04$), and PPI-increasing effects of AMPH in LE rats for the 20-ms prepulse interval ($p<0.03$). *Inset* shows no significant effects of AMPH on startle magnitude in SD or LE rats. **b** ANOVA revealed significant PPI-reducing effects of AMPH in rats with high baseline PPI (SD:LE=6:4; $p<0.035$), and PPI-increasing effects of AMPH in low-gating rats for the 10–20-ms prepulse intervals (SD:LE=4:6; $p<0.05$). *Inset* shows no significant effects of AMPH on startle magnitude in low- or high-gating rats

between personality scale scores and laboratory indices of AMPH sensitivity. Many suggestive correlations were detected (Table 2), but none that would remain significant after correcting alpha for multiple comparisons. While no differences in AMPH effects on subjective assessments were detected between individuals scoring low vs. high NS or SSS scores, it is possible that neither the dose of AMPH nor the choice of specific subjective scales were most sensitive for detecting such group differences (Kelly et al. 2006).

Rodent testing

ANOVA of PPI revealed no significant effect of strain ($F<1$) or AMPH ($F=1.29$, df 1, 18, ns), but a significant interaction of AMPH × strain ($F=7.33$, df 1, 18, $p<0.015$). There were also significant effects of prepulse interval ($F=33.79$, df 4, 18, $p<0.0001$) and interactions of interval × strain ($F=4.42$, df 4, 72, $p<0.005$) and interval × AMPH ($F=5.75$, df 4, 72, $p<0.0005$; Fig. 4a). Separate ANOVAs in SD and LE rats revealed significant PPI-reducing effects of AMPH in SD rats (main effect of AMPH: $F=5.92$, df 1, 9, $p<0.04$; AMPH × prepulse interval interaction ns), and significant PPI-increasing effects of AMPH at the 20-ms prepulse interval in LE rats (main effect of AMPH ns; AMPH × prepulse interval interaction: $F=4.61$, df 4, 36, $p<0.005$; post hoc comparison limited to 20 ms interval: significant effect of AMPH, $p<0.03$). AMPH had no significant effects on startle magnitude in either rat strain (main effects of strain, AMPH, and interaction all $F<1$).

Rats were then divided into "low gaters" vs. "high gaters", as had been done above for women, independent of rat strain. ANOVA of PPI revealed a near significant effect of "low" vs. "high" grouping ($F=3.50$, df 1, 18, $p<0.08$), no significant effect of AMPH ($F=1.27$, df 1, 18, ns), and a significant interaction of baseline PPI vs. AMPH ($F=7.00$, df 1, 18, $p<0.02$; Fig. 4b). Separate ANOVAs in low- and high-gating rats revealed significant PPI-reducing effects of AMPH in high-gating rats (main effect of AMPH: $F=6.44$, df 1, 9, $p<0.035$; AMPH × prepulse interval interaction ns), and significant PPI-increasing effects of AMPH at the 10–20-ms prepulse interval in low-gating rats (main effect of AMPH ns; AMPH × prepulse interval interaction: $F=4.78$, df 4, 36, $p<0.004$; post-hoc comparison limited to 10–20 ms intervals: significant effect of AMPH, $p<0.05$). AMPH had no significant effects on startle magnitude in either rat group (main effects of baseline PPI, AMPH, and interaction all $F<1$).

Strain distributions across the low (SD:LE=4:6) and high baseline PPI subgroups (SD:LE=6:4) were comparable. Because strain (unlike personality scale score) is a categorical variable and thus not informative as a covariate, both strain and baseline PPI were included as grouping factors in the ANOVA structure. The results revealed significant interactions of AMPH × strain ($p<0.03$) and AMPH × baseline PPI ($p=0.03$), and no significant interaction of strain × baseline PPI × AMPH ($F=1.42$, df 4, 64, ns). In other words, strain and baseline PPI conferred independent, significant effects on AMPH PPI sensitivity.

Discussion

AMPH has a rate-dependent effect on PPI in normal women: the impact of AMPH on PPI is significantly correlated with the amount ("rate") or baseline PPI. One might speculate that this pattern simply reflects a "regression to the mean", perhaps based on physiological ceiling or floor effects of PPI. However, PPI is a stable phenotype, with test–retest reliability exceeding 0.8 under conditions comparable to those tested here, for intervals ranging from 2 weeks to 1 year (Cadenhead et al. 1999; Swerdlow et al. 2001; Light et al. 2007); this would make it unlikely that subjects would systematically alternate "low" vs. "high" PPI status across tests. Furthermore, such an effect could not be explained by a physiological "ceiling" in the present data: "high" basal PPI in women is actually comparable to "low" basal PPI in men using the current stimulus parameters (Swerdlow et al. 2006), yet AMPH has opposite effects on PPI in these two groups. Simple "range-dependent" effect of AMPH on PPI also would not be consistent with the current data from low vs. high NS subjects. In this case, low vs. high NS groups do not differ in basal PPI levels, and yet AMPH has opposite effects on these groups, increasing PPI in low NS subjects, and reducing PPI in high NS subjects.

Rate-dependent effects on PPI have been reported in normal men with DA agonists (pergolide and amantadine; Bitsios et al. 2005) and DA antagonists (quetiapine: Swerdlow et al. 2006; haloperidol: Csomor et al. 2008; clozapine: Vollenweider et al. 2006). As in the present study, in some instances, these rate-dependent effects have impacted both PPI and prepulse facilitation ("PPF", i.e., "negative" PPI; e.g., Swerdlow et al. 2006). Thus, DAergic drugs (including AMPH) appear to reduce the magnitude-modulating impact of the prepulse on startle, whether this impact is to reduce (PPI) or increase (PPF) startle magnitude. Combined with the present AMPH data and our past report of comparable effects of AMPH in normal men (Swerdlow et al. 2003a), a consistent set of findings has emerged that suggests that DAergic manipulations modify prepulse effects on startle magnitude in normal humans in a manner that depends on basal levels of PPI.

The observation that the effects of AMPH on PPI are inversely related to baseline levels of PPI is not unique in the literature of AMPH effects: a similar phenomenon of "rate-dependent" drug action has been hypothesized to underlie some of the therapeutic effects of stimulants in attention deficit hyperactivity disorder (Lyon and Robbins 1975). Interestingly, an NIMH study (Fleming et al. 1995) reported that after AMPH challenge, cognitive function deteriorated in high NS subjects but improved in low NS subjects, and concluded, "some cognitive abilities of persons who may have relatively high DAergic tone are disrupted by AMPH, while those with relatively low DAergic tone may have their performance enhanced." Such "normalizing" effects of AMPH have been variously ascribed to its action on opposing neural systems (e.g.,

mesolimbic and nigrostriatal DA systems) or on DA transmission supported by different DA receptor subtypes, among other possible mechanisms.

The neural basis for these rate-dependent effects is not known. PPI levels in normal humans may reflect, in part, resting DAergic tone in the basal forebrain (cf. Swerdlow et al. 2003c). Some evidence suggests that rat strains with higher forebrain DA turnover and elevated COMT expression (e.g., LE rats) are most sensitive to the PPI-enhancing effects of DA agonists, while strains with lower forebrain DA turnover and COMT expression (e.g., SD rats) are most sensitive to the PPI-disruptive effects of DA agonists (Swerdlow et al. 2005; Shilling et al. 2008). This pattern was reproduced in the present study. Conceivably, similar neurochemical and genetic differences might distinguish low- vs. high-"gating" humans. Some evidence supporting such a mechanism is now emerging from studies of neurocognitive and genetic characteristics of low- vs. high-"gating" normals (Bitsios et al. 2006).

The notion that low vs. high levels of COMT activity might impact sensitivity to the PPI-disruptive effects of DA agonists might find support from the evidence that PPI AMPH sensitivity appears to be linked to personality measures of novelty seeking and sensation seeking, both in the present cohort of women and in mixed-gender samples (Hutchison et al. 1999). High novelty seeking in women has been linked to Met/Met Val158Met COMT polymorphism (Golimbet et al. 2007). Conceivably, low COMT activity associated with this Met/Met polymorphism and high NS scores might account for the predominant PPI-reducing effects of AMPH in women, while low novelty seeking linked to high COMT activity might account for predominant PPI-enhancing effects of AMPH. This identical pattern of PPI AMPH sensitivity is observed in rat strains with low vs. high COMT expression in the nucleus accumbens (present study, Fig. 4; Shilling et al. 2008). Such a mechanism might also account for the PPI-*enhancing* effects of the DA *antagonist*, quetiapine, in normal low-gating individuals with *high novelty seeking* traits (Swerdlow et al. 2006). In this case, individuals with high novelty seeking and low COMT activity may be exhibiting low basal PPI levels due to high basal DA tone, and these effects would be particularly sensitive to the PPI-enhancing effects of DA receptor blockade. One important caveat to the hypothesis that low COMT activity associated with the Met/Met polymorphism accounts for the prominent PPI-reducing effects of AMPH in high NS subjects is that the link between high NS and the Met/Met polymorphism was detected in one study *only in women* (Golimbet et al. 2007). Interestingly, in our previous report (Swerdlow et al. 2003b), high NS was not associated with greater PPI AMPH sensitivity in men (and in fact, the opposite pattern was detected). We hope to directly test the relationship between COMT polymorphisms, novelty seeking, and PPI AMPH sensitivity in future studies in humans.

Importantly, these relationships between NS, baseline PPI, and drug sensitivity were all detected in clinically normal individuals and are all presumed to reflect processes intrinsic to DAergic activity, up to the level of the post-synaptic DA receptor (in the case of antipsychotic effects on PPI). How these processes relate to PPI abnormalities in pathological populations, which may reflect abnormalities in cortical or subcortical systems "beyond" the DA neuron, is not easily discerned from the present data.

A potential limitation of the present rodent studies is the lack of hormonal measures: female rats were tested independent of their estrous cycle phase. Koch (1998) reported that PPI in female SD rats was reduced during proestrous compared to diestrous or estrous, but that sensitivity to the PPI-disruptive effects of a dopamine agonist did not vary across the estrous cycle. We previously reported a pattern of strain differences in PPI AMPH sensitivity among male SD vs. LE rats comparable to those detected in the present study with female rats (Swerdlow et al. 2003a), suggesting that the present findings are not mediated by strain differences in female reproductive hormones.

We do not know which DA receptor subtypes might be responsible for the "rate-dependent" and perhaps COMT-dependent effects of dopaminergic manipulations on PPI. The drugs with which one or both of these effects have been detected (amphetamine (Hutchison et al. 1999; Swerdlow et al. 2003b and present data), amantadine and pergolide (Bitsios et al. 2005) and several mixed D2-family antagonists (Swerdlow et al. 2006; Vollenweider et al. 2006; Csomor et al. 2008) do not distinguish neatly among D2, D3, or D4 receptors. We are currently testing the relationship between basal PPI levels, novelty seeking, and the effects of the D3-preferential agonist, pramipexole, on PPI in normal men. Interestingly, the PPI-disruptive effects of pramipexole do not differ between rat strains with low vs. high accumbens COMT activity (Weber et al. 2008). If our cross-species model is valid as it relates narrowly to the role of COMT in the D3 regulation of PPI, we would predict that—in contrast to AMPH—pramipexole effects on PPI should not differ between individuals with low vs. high novelty seeking traits.

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