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Heritable Differences in the Dopaminergic Regulation of Behavior in Rats: Relationship to D_2-Like Receptor G-Protein Function

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We reported heritable differences between Sprague–Dawley (SD) and Long Evans (LE) rats in their sensitivity to the disruption of prepulse inhibition of startle (PPI) by dopamine (DA) agonists, and in their basal levels and turnover of forebrain DA. In an effort to better understand these differences, we assessed strain patterns in the efficacy of D_2-like receptor-G-protein coupling using [^{35}S]GTPγS binding in brain regions that contribute to the dopaminergic regulation of PPI. Sensitivity to the PPI-disruptive effects of apomorphine (APO) was examined in SD, LE, and F1 (SD × LE) rats. Basal and DA-stimulated [^{35}S]GTPγS binding were then assessed in these rats using conditions that preferentially exclude Gs proteins to favor visualization of D2-like receptors. To explore the behavioral specificity of these strain differences, locomotor responses to APO and amphetamine (AMPH) were also assessed in SD, LE, and F1 rats. Strain differences were evident in the PPI-disruptive effects of APO (SD > F1 > LE), and in the locomotor responses to AMPH (LE > F1 > SD) and APO (SD exhibited motor suppression, LE exhibited motor activation). Compared to SD rats, LE rats exhibited greater DA-stimulated [^{35}S]GTPγS binding in nucleus accumbens and caudatoputamen, while F1 progeny had intermediate levels. In conclusion, SD and LE rats exhibit heritable differences in D2-mediated behavioral and biochemical measures. Conceivably, genes that regulate heritable differences in forebrain D2 function may contribute to heritable differences in PPI in patients with specific neuropsychiatric disorders, including schizophrenia and Tourette Syndrome.
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INTRODUCTION

The neural and genetic bases for neuropsychiatric disorders may in some cases be studied most easily via surrogate or intermediate phenotypes. Several neuropsychiatric disorders, including schizophrenia (Braff *et al*, 1978) and Tourette Syndrome (TS) (Castellanos *et al*, 1996), are characterized by a loss of sensorimotor gating, as measured by prepulse inhibition of the startle reflex (PPI). By understanding the neural and genetic regulation of PPI, it might be possible to gain insight into the biology of the more complex clinical phenotypes found in these disorders.

PPI is a cross-species measure of sensorimotor gating, defined as the reduction in startle amplitude that occurs when the startling stimulus is preceded 30–500 ms by a barely audible sensory event or 'prepulse' (Graham, 1975). PPI is diminished in several neuropsychiatric disorders, including schizophrenia and TS (cf. Braff *et al*, 2001); in rats, PPI is disrupted by dopamine (DA) agonists, including the direct DA agonist, apomorphine (APO), and the indirect DA agonist, amphetamine (AMPH) (Swerdlow *et al*, 1986; Mansbach *et al*, 1988). Heritable differences in PPI sensitivity to DA agonists have been identified in outbred rat strains (Swerdlow *et al*, 2003, 2004a, b). For example, crosses and backcrosses between Harlan Sprague–Dawley (SD) and Harlan Long Evans (LE) rats revealed an orderly pattern of PPI APO sensitivity (SD > N2 > F1 > LE), suggestive of the additive effects of a relatively small number of genes (Swerdlow *et al*, 2004a, b).

We recently reported that SD rats had generally lower basal levels of striatal DA turnover compared to LE rats, but that SD and LE rats did not differ significantly in their neurochemical response to APO or AMPH (Swerdlow *et al*, 2005). In an effort to further understand the biochemical basis for SD *vs* LE strain differences in PPI DA agonist

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sensitivity, we examined the efficacy of D_2-like receptor-G-protein coupling in SD, LE, and F1 (SD × LE) rats. The PPI behavioral phenotype was confirmed, and the behavioral specificity of this phenotype was explored using measures of DA agonist-induced changes in locomotor activity in SD, LE, and F1 rats.

MATERIALS AND METHODS

Animals

Male SD ($n = 12$) and LE ($n = 12$) rats were obtained as adults from commercial suppliers (Harlan Laboratories; SD: San Diego, CA; LE: Indianapolis, IN). Subjects from these strains were either tested for APO sensitivity, or were reciprocally crossed (with representation of both sexes from both strains) to produce F1 litters, which were allowed to mature to adulthood prior to testing (only male F1s were tested; $n = 22$). Rats received food and water *ad libitum* while housed in a climate-controlled facility with reverse 12-h light/dark cycle. All behavioral testing took place in the dark phase. Rats were handled within 48 h of arrival and allowed to acclimate to the laboratory for 7 days prior to behavioral testing.

Prepulse Inhibition Testing

Startle chambers (SR-LAB Startle Reflex System; San Diego Instruments) were located in a sound-attenuated room with 60 dB ambient noise. Rats were exposed to a brief 'matching' startle session used to assign rats to balanced drug groups according to their average level of PPI. Testing continued 4 days later. All animals received either APO (0.5 mg/kg, s.c.) or vehicle (0.1% ascorbic acid) immediately prior to PPI testing. Test sessions were approximately 19 min long and consisted of 5 min of 70 dB background followed by five trial types: PULSE (120 dB(A) 40 ms noise burst), prepulse trials (20 ms noise burst 5, 10, or 15 dB above background followed 100 ms later by PULSE), and NOSTIM trial. After 3 days, the test was repeated, with dose reversed and treatment order balanced within and between rat strain groups. Thus, APO dose was a within-subject variable.

Locomotion

In separate rats ($n = 75$), locomotor activity and stereotypy ratings were recorded. Locomotor activity was measured using wire-mesh photocell cages (22 × 35 × 15 cm) fitted with two parallel infrared beams 1 cm above the floor, perpendicular to the long axis of the cage. The total number of beam breaks and crossovers (sequential interruption of separate beams) were calculated for each 10 min interval during a 90 min test. Rats were habituated to the cages for 90 min 4 days prior to their first test. On test days, rats were placed in the activity chambers for a 60 min acclimation period, removed, and injected with AMPH (0, 0.75, 1.5, 4.5 mg/kg, s.c.) and then 6 days later, retested with APO (0, 0.5, 2.5, or 7.5 mg/kg, s.c.) with drug dose order balanced across rat strains. Blind behavioral ratings recorded over a 60 min period at 20 min intervals identified the following behaviors: asleep, sniffing, locomotion, rearing, grooming, licking, gnawing, and 'head down'; raters were unaware of the distinction of F1 *vs* LE rats, and drug dose for all rats. Thus, both AMPH dose and APO dose were between-subject variables, in separate analyses.

[^{35}S]GTPγS Binding Analysis

After PPI testing, animals were decapitated and their brains were rapidly frozen in 2-methylbutane at −35°C, then stored at −80°C. Brains were sectioned using a −20°C cryostat at 16 μm, serial sections were collected starting at a level corresponding to 1.6 mm anterior to bregma (Paxinos and Watson, 1997) and thaw-mounted onto SuperFrost Plus slides, and slides were stored at −80°C prior to processing.

[^{35}S]GTPγS binding was preformed as described previously (Culm *et al*, 2003; Culm and Hammer, 2004). Sections were preincubated in assay buffer (50 mM Tris-HCl, 2 mM MgCl, 0.2 mM EGTA, 100 mM NaCl, and 0.2 mM DTT pH 7.4) for 15 min at 25°C followed by a 15 min incubation in the same buffer with the addition of 2 mM GDP (ICN; Costa Mesa, CA). Sections were then incubated in assay buffer containing 2 mM GDP and 50 pM [^{35}S]GTPγS (NEN-Perkin–Elmer Life Sciences, Boston, MA) in the absence (basal) or presence of 100 mM DA (Sigma-Aldrich; St Louis, MO) for 1 h at 25°C. These assay conditions utilizing a low Mg^{2+} concentration favor labeling of G_i proteins (coupled to D_2-like receptors), because G_s activation requires much higher Mg^{2+} concentration (25–50 mM; Waeber and Moskowitz, 1997). Furthermore, DA-stimulated [^{35}S]GTPγS binding is blocked by the D_2-like receptor antagonist, raclopride, but unaffected by the D_1-like antagonist, SCH 23390 (Culm *et al*, 2003). After incubation, sections were washed three times in ice-cold 50 mM Tris-HCl, (pH 7.4) and once in ice-cold distilled water. After slides were allowed to dry overnight, they were exposed to X-ray film (Biomax MR, Eastman Kodak Company, Rochester, NY) for 3 days. The relative amount of [^{35}S]GTPγS binding was determined using a calibration curve based on ^{14}C radiostandards (ARC-146; American Radiolabeled Chemicals St Louis, MO) which were coexposed on the film.

Quantitative autoradiographic analysis of [^{35}S]GTPγS binding was conducted in the NAc core and shell, dorsolateral (DL) and medial (Med) caudatoputamen, and cingulate and somatosensory cortices (Figure 1). In all cases, measurements were taken bilaterally from at least three adjacent coronal sections that were randomly selected at a level approximately 1.2 mm anterior to bregma without knowledge of the rat strain or binding condition. [^{35}S]GTPγS binding was also measured in cortical laminae; cingulate cortex was separated into superficial and deep regions, while layers II/III, IV, V, and VI of somatosensory cortex were distinguished by differences in optical density and analyzed separately. Autoradiographic images were analyzed using NIH ImageJ (developed by Wayne Rasband, NIMH; available on the Internet at http://rsb.info.nih.gov/nih-image/). Mean data were calculated for both basal and DA-stimulated binding in each region of each animal, and the percent binding above basal was then calculated.

Coat Pigment Phenotype

The most visible phenotypic difference between albino SD and hooded LE rats is their coat pigmentation. This categorical phenotype has an autosomal dominant inheritance, with 100% of F1s and 50% of F2s exhibiting hooded patterns (Swerdlow *et al*, 2004b). However, the amount of pigmented fur area is inherited in a graded pattern (LE > F1 > hooded N2 pigmented fur area), and in N2s, this area correlated significantly with APO PPI sensitivity (Swerdlow *et al*, 2004b). In the present study, this phenotype was quantified in all LE and F1 rats used in startle and GTPγS studies, by calculating the proportion of dorsal surface area (excluding the head, which is completely black in all hooded rats) with black pigmentation. Photographs were taken using a Canon PowerShot A20 Digital Camera (Model PC1007, 2.1 Megapixels, 3X Zoom Lens), exposure value (EV) = 7.65, determined by a LUNA-PRO F light meter (Grossen, Germany). Each rat was positioned vertically by an experimenter, with its ventrum against a white background, by securing its head in the left hand and its tail in the right hand. The experimenter was unaware of the behavioral data of each rat.

Images were transferred to a Macintosh G4 computer, and .jpg files opened in NIH Image v. 1.62. The dorsal surface area of each rat was normalized based on a standardized cross-sectional distance of approximately 4.83 cm measured at the lateral extent of the pelvic rim (to correct for individual differences in body size). Units were set at pixels/cm and the scale for each photo was adjusted so that all lengths would have less than a 1.2% variability. Total dorsal black surface area (in corrected square centimeter) was calculated for each rat by NIH Image software from free-hand tracings by an experimenter who was blind to the behavioral results.

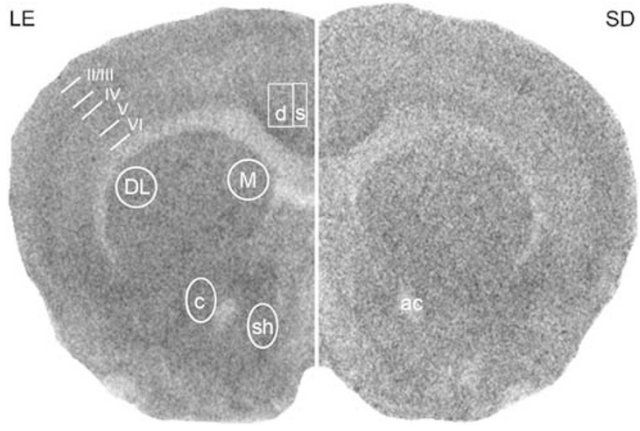

Figure 1 Autoradiographs showing DA-stimulated [^{35}S]GTPγS binding in sections from LE (left) and SD (right) rat brains. The relative size and location of the brain regions assessed is shown. Abbreviations: ac, anterior commissure; c, nucleus accumbens core; d, cingulate cortex, deep; DL, dorsolateral caudatoputamen; M, medial caudatoputamen; s, cingulate cortex, superficial; sh, nucleus accumbens shell. Roman numerals label somatosensory cortex layers II/III, IV, V, and VI, with lines drawn between layers in the region where labeling was assessed.

Statistics

PPI was defined as 100−((startle amplitude on prepulse trials/startle amplitude on PULSE trials) × 100), and was analyzed by mixed-design ANOVAs. Any significant drug effects on %PPI prompted separate analyses to assess the relationship of these effects to drug-induced changes in startle magnitude on PULSE and prepulse trials. All startle data were analyzed using an ANOVA with strain as a between-subject factor and dose, trial block, and trial type as within-subject repeated measures. Relevant ANOVA values are shown in Table 1. A measure of drug 'effect' (mean PPI after vehicle minus mean PPI after APO) was

Table 1 ANOVA Results for Startle Measures

Measure	Factor	ANOVA statistics	Relevant *post hoc* comparisons
%PPI	Strain	$F = 6.42$, df 2,41, $p < 0.004$	
	Dose	$F = 317.78$, df 1,41, $p < 0.0001$	
	Strain × dose	$F = 29.97$, df 2,41, $p < 0.0001$	
	Intensity	$F = 97.23$, df 2,82, $p < 0.0001$	
	Strain × dose × intensity	$F < 1$	
%PPI vehicle	Strain	$F = 1.93$, df 2,41, NS	
PPI APO effect	Strain	$F = 29.97$, df 2,41, $p < 0.0001$	SD > F1; F1 > LE; SD > LE
PULSE magnitude	Strain	$F = 5.04$, df 2,41, $p < 0.015$	F1 > SD; F1 > LE
	Dose	$F = 22.48$, df 1,41, $p < 0.0001$	
	Strain × dose	$F = 2.26$, df 2,41, NS	
PULSE APO effect	Strain	$F = 2.26$, df 2,41, NS	
Habituation	Strain	$F = 12.85$, df 2,41, $p < 0.0001$	
	Dose	$F = 10.62$, df 1,41, $p < 0.0001$	
	Trial block	$F = 156.92$, df 1,41, $p < 0.0001$	
	Strain × dose	$F = 1.16$, df 2,41, NS	
	Strain × trial block	$F = 10.19$, df 2,41, $p < 0.0005$	Block 1: F1 > SD; F1 > LE
	Strain × dose × trial block	$F = 1.77$, df 2,41, NS	

also calculated and compared across strains; this value has previously been shown to be very sensitive to differences across strains and generations in studies using APO (Swerdlow *et al*, 2002, 2004b). Photocell beam breaks and crossovers were analyzed by ANOVA, with strain and dose as between-subject factors and time as the within-subject factor. For both startle and locomotor activity, *post hoc* comparisons of significant interactions and relevant main factor effects were conducted using Fisher's Protected Least Significant Difference (PLSD) and one-factor ANOVA tests. Behavioral ratings were recorded as 'present' or 'absent' and SD *vs* LE comparisons for each drug dose and time point were made by χ^2. No significant differences in the main behavioral measures were detected based on maternal strain of F1 rats (SD *vs* LE), and thus this variable was not used as a grouping factor. For measures of basal and DA-stimulated [^{35}S]GTPγS binding, three separate ANOVAs were used, with three brain regions grouped into specific subregions: 'striatum' (NAC core, NAC shell, dorsolateral striatum, medial striatum), cingulate cortex (deep and superficial), and primary somatosensory cortex (layers II/III, IV, V, and VI); strain (SD, LE, F1) was the between-subject factor. Simple regressions were used to assess associations between DA-stimulated [^{35}S]GTPγS binding and other phenotypes. Based on our past behavioral findings (Swerdlow *et al*, 2004b), specific comparisons with F0 and F1 strains were planned *a priori*, with the following simple 'additive' model predictions: (1) SDH and LEH sensitivity would differ by the largest magnitude, (2) F1 sensitivity would be intermediate between parental strains. Since this intermediate sensitivity would be predicted to diminish the main and interaction effects of strain by ANOVA, some analyses were initially limited to SD and LE strains α was 0.05.

RESULTS

PPI and Startle

ANOVA of PPI (Figure 2a) revealed a significant main effect of strain ($p<0.004$) and APO dose ($p<0.0001$), and a significant strain × dose interaction ($p<0.0001$), in addition to other significant main and interaction effects (Table 1). *Post hoc* comparisons revealed no differences in vehicle levels of PPI across strains, but analysis of the APO effect on PPI (PPI after vehicle minus PPI after APO) revealed a significant effect of strain ($p<0.0001$) (SD > F1: $p<0.003$; F1 > LE: $p<0.0001$; SD > LE: $p<0.0001$).

Analyses of startle magnitude on PULSE trials (Figure 2a, inset) revealed significant main effects of strain ($p<0.015$) and dose ($p<0.0001$), but no significant strain × dose interaction. Distinct from patterns of PPI, startle magnitude was comparable in SD and LE rats, but elevated in F1 rats. Analysis of startle magnitude on PULSE and prepulse trials revealed that APO eliminated the ability of prepulses to inhibit startle in SD rats, while prepulses retained this effect in LE and F1 rats. As APO increased startle magnitude in all strains, separate comparisons among subgroups with the least *vs* greatest APO-potentiated startle revealed the independence of APO effects on startle and PPI in all strains (Table 1; Figure 2b). The main effect of strain on APO PPI effect remained statistically significant when APO effects on PULSE startle magnitude was used as a covariate

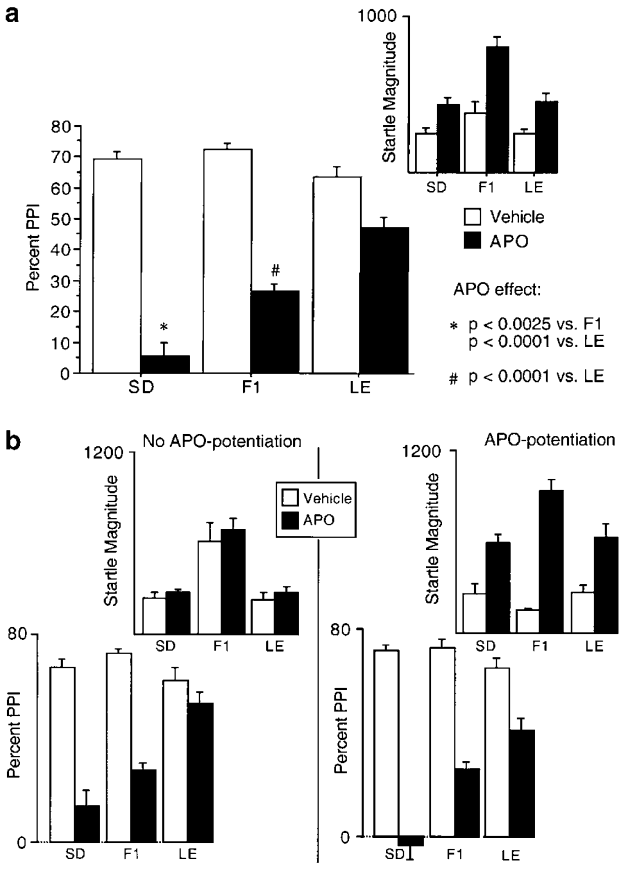

Figure 2 Effects of APO on PPI in SD, F1, and LE rats. (a) SD > F1 > LE gradient in APO effect on PPI. Inset shows F1 > SD = LE effects of APO on startle magnitude on PULSE trials. (b) SD > F1 > LE pattern of APO PPI sensitivity is independent of APO effects on startle magnitude. Rats in each strain are divided based on median split of APO potentiation of startle magnitude (insets). Left figures show subsample with no APO potentiation of startle magnitude (see inset); right figures show subsample with large APO potentiation of startle magnitude (see inset). Note that both subsamples show pattern of SD > F1 > LE sensitivity to APO disruption of PPI.

(F = 4.87, df 2,38, $p<0.015$), despite the fact that PULSE startle magnitude is an element of the equation used to calculate percent PPI.

Startle habituation was most pronounced in F1 rats, reflecting significantly greater startle magnitude in the initial trial block (F1 > SDH: $p<0.0001$; F1 > LEH: $p<0.0003$; SDH *vs* LEH: NS); there were no strain differences in APO effects on habituation (Table 1). Analysis of motor activity during NOSTIM trials revealed the previously reported APO-induced increase in NOSTIM activity ($p<0.03$), which was greater in SD *vs* LE rats ($p<0.002$). Interestingly, APO effects on NOSTIM activity did not correlate with APO PPI effects within rats ($R=-0.23$, NS); subgroups of SD, LE, and F1 rats with no significant APO effects on NOSTIM activity or strain × dose NOSTIM differences still exhibited robust PPI-disruptive effects of APO ($p<0.0001$) and strain differences in this effect (strain × dose interaction: $p<0.0001$). The main effect of strain on APO PPI effect remained highly significant when APO NOSTIM effect was used as a covariate (F = 15.98, df 2,38, $p<0.0001$).

As we observed previously (Swerdlow *et al*, 2004b), coat pigmentation area was significantly greater in LE than F1 rats ($F = 64.18$, df 1,32, $p < 0.0001$). A significant negative correlation was detected between coat pigmentation area and APO PPI effect ($p < 0.0001$) (Figure 3), but not between coat pigmentation and APO effects on PULSE amplitude or NOSTIM activity. As in our previous report (Swerdlow *et al*, 2004b), coat pigmentation did not correlate significantly with any startle variable in vehicle-treated rats.

[^{35}S]GTPγS Binding

Basal [^{35}S]GTPγS binding did not differ across strains in any brain region (all $Fs < 1$; Table 2). In contrast, in every brain region, there was a pattern of greater DA-stimulated [^{35}S]GTPγS binding in LE *vs* SD rats, with F1 rats exhibiting intermediate binding levels (Figure 4). ANOVA revealed that this pattern achieved statistical significance for DA-stimulated [^{35}S]GTPγS binding in striatum ($F = 4.77$, df 2,35, $p < 0.015$; Figure 4a), but not in cingulate ($F = 1.86$) nor in primary somatosensory cortex ($F < 1$; Figure 4b). In striatum, DA-stimulated [^{35}S]GTPγS binding was significantly greater in LE *vs* SD rats ($p < 0.006$), with F1 rats exhibiting intermediate values (F1 *vs* LE, $p = 0.05$). This pattern was evident and statistically significant in each of the four striatal subregions.

Simple regression analyses revealed statistically significant negative correlations between DA-stimulated [^{35}S]GTPγS binding in all regions and APO effects on PPI, including striatum ($R = -0.49$; $p < 0.002$), cingulate cortex ($R = -0.44$, $p < 0.008$), and primary somatosensory cortex ($R = -0.42$, $p < 0.01$) (Figure 5). A weak but significant correlation was detected between APO effects on startle magnitude on PULSE trials and DA-stimulated [^{35}S]GTPγS binding in cingulate cortex ($R = -0.35$, $p < 0.04$), but not in either striatum or somatosensoy cortex. APO effects on NOSTIM activity did not correlate significantly with DA-stimulated [^{35}S]GTPγS binding in any brain region ($R = -0.09$, -0.04, and -0.04 for striatum, cingulated, and somatosensory cortex, respectively). Among LE and F1 rats, regression analyses revealed a nonsignificant trend towards a positive correlation between fur pigmentation area and DA-stimulated [^{35}S]GTPγS binding in striatum ($R = 0.30$; Figure 5, inset), but not cingulate ($R = 0.17$) or somatosen-

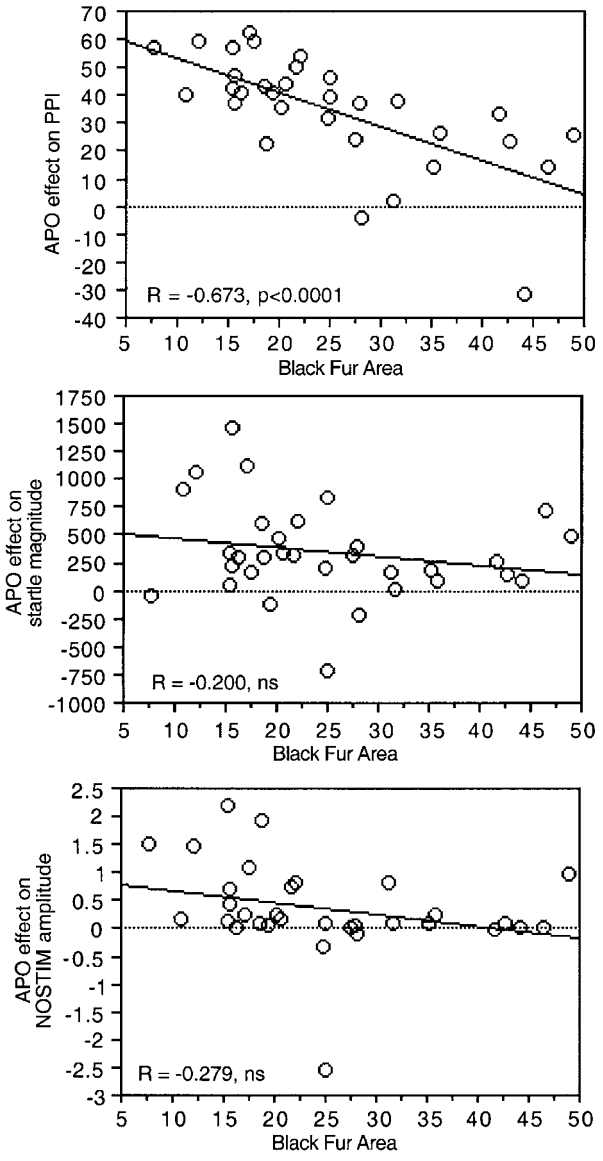

Figure 3 Correlations of black fur pigmentation area in LE and F1 rats *vs* APO effect on PPI (top: PPI after vehicle minus PPI after APO; $p < 0.0001$), startle magnitude on PULSE trials (middle: startle magnitude after APO minus startle magnitude after vehicle; NS) and NOSTIM levels (bottom: NOSTIM after APO minus NOSTIM after vehicle; NS).

Table 2 Basal [^{35}S]GTPγS Binding (nCi/g; Mean (SEM))

	NAC		Striatum		Cingulate cortex		Somatosensory cortex layers			
Strain	**Core**	**Shell**	**DL**	**Medial**	**Deep**	**Superficial**	**II/III**	**IV**	**V**	**VI**
SD	0.59	0.51	0.59	0.56	0.66	0.52	0.44	0.57	0.49	0.54
(SEM)	0.09	0.08	0.10	0.08	0.11	0.09	0.07	0.10	0.09	0.09
F1	0.64	0.58	0.68	0.66	0.68	0.55	0.44	0.59	0.51	0.56
(SEM)	0.07	0.07	0.08	0.07	0.10	0.09	0.06	0.08	0.07	0.08
LE	0.58	0.50	0.62	0.58	0.62	0.50	0.45	0.57	0.50	0.55
(SEM)	0.10	0.09	0.11	0.10	0.13	0.10	0.08	0.10	0.09	0.10

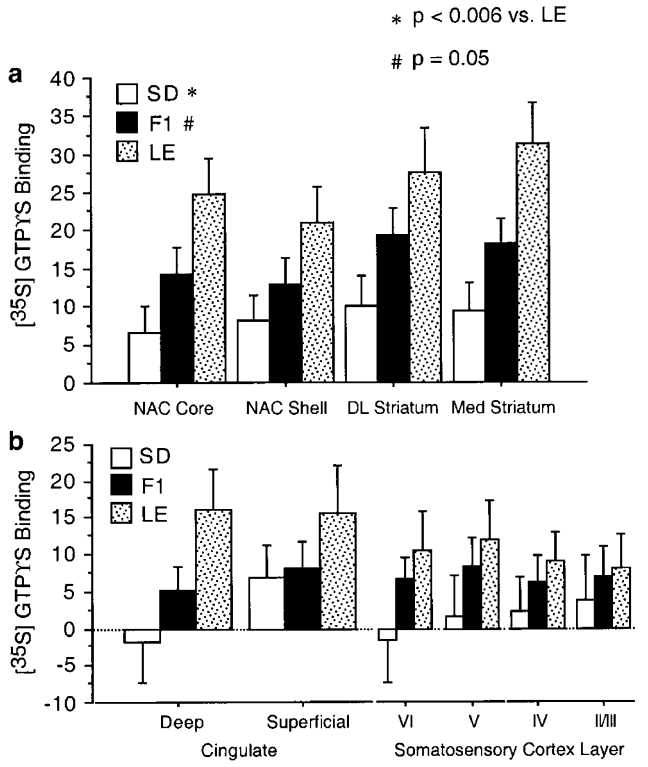

Figure 4 [^{35}S]GTPγS binding in different brain regions in 38 rats (SD: $n=11$; LE: $n=10$; F1: $n=17$). (a) Binding in striatum (NAC core and shell, DL and Medial striatum) reveals significant SD < F1 < LE gradient. (b) Binding in cingulate cortex (left) and somatosensory cortex (right) reveals no significant strain differences, although deeper laters of both regions exhibit similar pattern to that observed in striatum.

sory cortex ($R=0.06$). DA-stimulated [^{35}S]GTPγS binding did not correlate significantly with any startle variable in vehicle-treated rats.

Locomotor Activity

To assess the behavioral specificity of strain differences in DA agonist sensitivity, locomotor activity was assessed in SD, LE, and F1 rats, after treatment with the indirect DA agonist, AMPH, or the direct DA agonist, APO. The locomotor response to AMPH was significantly more robust in LE than SD rats; F1 rats exhibited an intermediate response to some (1.5 mg/kg) but not other (0.75 or 4.5 mg/kg) AMPH doses (Figure 6a). ANOVA of pre-drug activity revealed no significant main effects of time or dose group (ie the dose assigned, but not yet given), a significant effect of time ($p<0.0001$) and strain $\times$ time interaction ($p<0.025$), but no other significant interaction effects. After AMPH, ANOVA revealed significant main effects of strain ($p<0.002$), dose ($p<0.0001$), and time ($p<0.0001$), and significant interactions of strain $\times$ dose ($p<0.001$) and dose $\times$ time ($p<0.0001$). Most AMPH-stimulated behavioral patterns were similar across strains, showing 'inverted U-shaped' dose functions. Compared to LE rats, more SD rats exhibited rearing in response to the 4.5 mg/kg dose of AMPH (40 min post-AMPH; $\chi^2=5.33$, $p<0.025$; 60 min post-AMPH; $\chi^2=8.57$, $p<0.01$), with F1s exhibiting an intermediate sensitivity.

In response to APO, SD rats exhibited a pronounced, dose-dependent suppression of locomotor activity, while LE rats exhibited significant locomotor activation, and F1 rats exhibited an intermediate APO response (Figure 6b). ANOVA of pre-drug activity revealed no significant effects of strain or dose group, a significant effect of time ($p<0.0001$), but no significant interactions. Analysis of post-APO activity revealed no significant main effect of APO or strain ($0.05<p<0.065$), or strain $\times$ APO interaction ($0.05<p<0.10$), a significant effect of time ($p<0.0001$), and significant interactions of strain $\times$ time ($p<0.025$) and APO $\times$ time ($p<0.015$). The strain $\times$ APO interaction reached statistical significance ($p<0.035$) when the analysis was limited to vehicle *vs* the highest APO dose (7.5 mg/kg).

Compared to SD rats, more LE rats exhibited sniffing in response to the 2.5 and 7.5 mg/kg doses of APO (20 min post-APO; $\chi^2=5.33$, $p<0.025$ both comparisons), but fewer exhibited rearing in response to the 2.5 mg/kg dose of APO (20 min post-APO; $\chi^2=5.33$, $p<0.05$; 40 min post-APO, $\chi^2=8.57$, $p<0.01$), and gnawing in response to 7.5 mg/kg APO (20 min post-APO; $\chi^2=5.33$, $p<0.025$). For each of these comparisons, F1 rats exhibited intermediate frequencies between those exhibited by SD and LE rats.

DISCUSSION

The present studies confirm that outbred Harlan SD and LE rats can be distinguished based on their behavioral sensitivity to DA agonists, and that the SD $\times$ LE F1 generation exhibits an intermediate phenotype related to this sensitivity. Our data on PPI APO sensitivity replicate previous reports (Swerdlow *et al*, 2003, 2004a, b, 2005), which also demonstrated that the N2 generation (F1 $\times$ SD) exhibited sensitivity to the PPI-disruptive effects of both APO and AMPH that was intermediate between F1 and SD strains. Thus, the present findings are consistent with the notion that sensitivity to the PPI-disruptive effects of DA agonists is a heritable phenotype among SD and LE strains.

The neural basis for this heritable phenotype was explored by examining strain-specific patterns of DA-stimulated [^{35}S]GTPγS binding among SD, LE, and F1 rats. Interestingly, significant SD *vs* LE differences were observed in DA-stimulated (but not basal) [^{35}S]GTPγS binding, with greater striatal DA-stimulated [^{35}S]GTPγS binding in LE than SD rats. As with the PPI-sensitivity phenotype, F1 rats exhibited an intermediate value, suggesting that this G-protein phenotype may also be heritable. However, the direction of the strain differences in DA-stimulated [^{35}S]GTPγS binding was *opposite* to those observed in PPI DA agonist sensitivity, and correlational analyses revealed that in each brain region (striatum, cingulum, and cortex), *greater* DA-stimulated [^{35}S]GTPγS binding predicted *less sensitivity* to the PPI-disruptive effects of APO.

A similar strain 'gradient' (SD < F1 < LE) for [^{35}S]GTPγS binding was observed in cingulate cortex and somatosensory cortex—particularly the deeper layers—as was observed in striatum. Levels of basal and DA-stimulated [^{35}S]GTPγS binding were much lower in cortical *vs* striatal regions, and strain differences in cortical DA-stimulated [^{35}S]GTPγS binding did not reach statistical significance; nonetheless, the strain patterns in deeper cortical layers

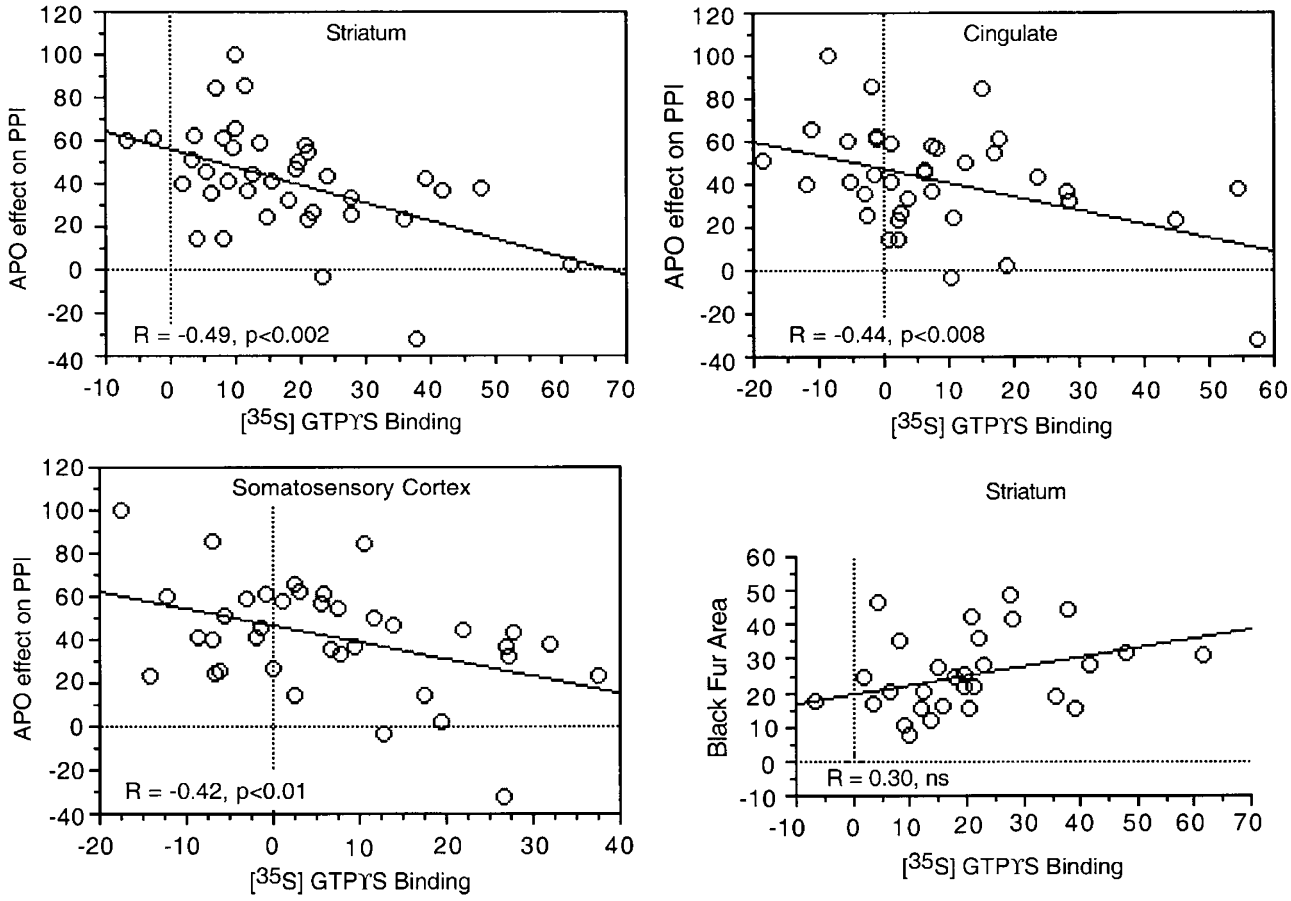

Figure 5 Correlations of [^{35}S]GTPγS binding in striatum ($p<0.002$), cingulate cortex ($p<0.008$), and somatosensory cortex ($p<0.01$) *vs* APO effect on PPI. Inset at lower right shows trend for positive correlation ($r=0.30$) of striatal [^{35}S]GTPγS binding with fur pigmentation area in LE and F1 rats.

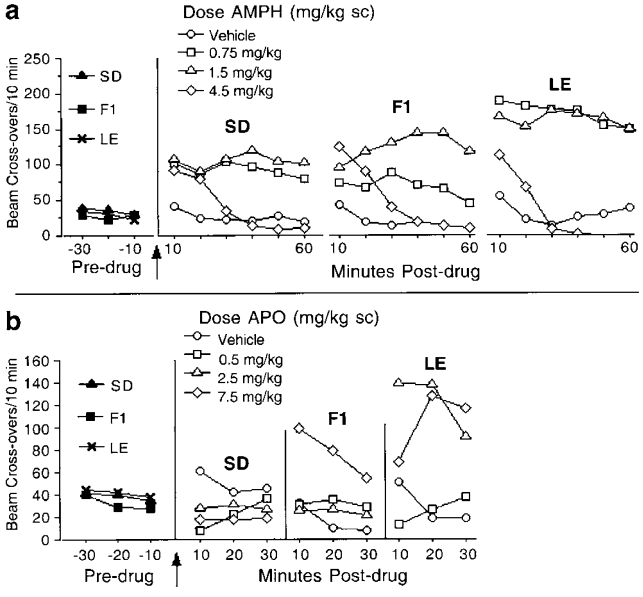

Figure 6 Locomotor activity (photobeam crossovers) in SD, F1, and LE rats (total $n=75$) prior to drug and after s.c. injection of one of four doses of AMPH (top: 10–60 min postinjection) or APO (bottom: 10–30 min postinjection).

(Figure 4b) suggest that genetic differences in G-protein regulation across these strains may not be regionally specific. This notion is further supported by the significant negative correlations between PPI APO sensitivity and DA-stimulated [^{35}S]GTPγS binding in cortex, as well as striatum.

There are at least three areas of potential weakness in these findings. First, the use of multiple correlations and ANOVAs across 3 different brain regions without correction of α raises the potential for false positive findings. However, even the weakest of these significant correlations ($p<0.01$) remained significant after Bonferonni correction ($\alpha=0.0167$). Second, the samples were not adequately large to examine distributional properties of the data in F1 rats, which might identify meaningful subgroups or correlations across phenotypes in this intermediate strain. Some subgroup analyses (eg F1s with SD *vs* LE mothers) failed to detect significant differences in the various phenotypes. Other findings in F1 rats (eg 'trend' positive correlation of fur pigmentation *vs* DA-stimulated [^{35}S]GTPγS binding) suggest that analyses in larger samples might be informative. Third, to allow full dose–response analyses, a separate sample of rats was used for analyses of strain differences in locomotor-activating effects of AMPH and APO *vs* analyses of PPI and [^{35}S]GTPγS binding. This precluded correlational

analyses of locomotor responses with either PPI sensitivity or [^{35}S]GTPγS binding. Nonetheless, the strains used in locomotor studies were acquired (SD, LE) or generated (F1) in the same laboratory and over the same time period as the rats used in startle/biochemical analyses. Thus, there is no reason to suspect that genetic drift or other substantive differences might exist between the strain categories used in these different studies.

Coat pigmentation was another quatifiable phenotype that distinguished SD, LE, and F1 rats. The mechanism responsible for a lack of pigmentation in albino rats is a genetic malfunction of tyrosinase in melanocytes (Searle, 1990), which interferes with melanin production. At least some albino rat strains also lack neuromelanin, potentially reflecting a lack of brain tyrosinase. Tyrosinase is present in both the human and rat central nervous system, in dopaminergic regions that regulate PPI (Miranda *et al*, 1984; Tief *et al*, 1998), and intracerebral infusion of tyrosinase results in increased straiatal DA release (Amicarelli *et al*, 1999). Conceivably, reduced brain tyrosinase activity in albino rats might be associated with reduced basal DA turnover in albino SD *vs* hooded LE rats (Swerdlow *et al*, 2005). At the least, the significant negative correlation ($p<0.0001$) between fur pigmentation and APO PPI effect noted in this study (and the apparent, although substantially weaker, relationship between pigmentation and striatal [^{35}S]GTPγS binding) suggests an association between physiological markers with connections to brain DA function, which may reflect overlapping genetic determinants.

Based on our observation that basal striatal DA turnover was significantly greater in Harlan LE *vs* SD rats (Swerdlow *et al*, 2005), it is worth considering the potential impact of basal DA activity on DA-stimulated [^{35}S]GTPγS binding. In albino Wistar rats, it has been reported that DA-stimulated [^{35}S]GTPγS binding can be modified by changes in basal DA activity. Thus, unilateral striatal DA depletion results in a small but significant increase in DA-stimulated [^{35}S]GTPγS binding ipsilateral to the depletion, evident 1 week post-lesion (Geurts *et al*, 1999). Apparently, the physiological effects of lower basal DA turnover on DA-stimulated [^{35}S]GTPγS binding cannot be easily equated to those associated with unilateral DA depletion: between SD and LE strains, the strain with lower basal DA turnover (SD) also exhibited lower DA-stimulated [^{35}S]GTPγS binding.

While strain-related patterns of DA-stimulated [^{35}S]GTPγS binding were opposite to what would have been predicted based on strain differences in PPI DA agonist sensitivity, and perhaps in basal DA turnover, they were largely consistent with the observed strain differences in DA agonist sensitivity for locomotor activation. While the pattern of specific behavioral changes is more complex than this, a general assessment is that compared to LE rats, SD rats exhibited less motor activation in response to AMPH, and more motor suppression in response to APO, while F1 rats generally exhibited intermediate values. This pattern of drug sensitivity could be viewed as consistent with the observed LE > F1 > SD pattern of striatal DA-stimulated [^{35}S]GTPγS binding, but is somewhat at odds with our previous failure to detect parallel strain differences in AMPH- or APO-stimulated striatal DA levels or turnover, over the same drug dose ranges and time courses (Swerdlow *et al*, 2005). We have previously reported that SD rats are more sensitive to the PPI-disruptive effects of APO, but less sensitive to the locomotor-activating effects of AMPH, compared to Wistar rats from the same supplier (Harlan Laboratories) (Swerdlow *et al*, 2000).

The process by which increased striatal DA-mediated signaling is translated to lower motor circuitry involves several changes downstream from the DA receptor (Swerdlow *et al*, 2001), and engages mechanisms that feed back to the striatum via striato-nigral and other recurrent loops. Activity within striatal efferent systems is balanced across D1 and D2 receptor-mediated signaling, direct and indirect output pathways, and other organizational properties of these circuits, including pre- *vs* postsynaptic DA receptor functions (Stoof and Kebabian, 1981; cf Gerfen, 2000; cf Graybiel, 2004). Activity at one level of this circuitry that blunts DA-mediated changes in one process may also trigger compensatory events that enhance the sensitivity of this circuit to DAergic activation of a second process (eg Koob *et al*, 1984). Furthermore, increased DA receptor sensitivity at two different levels of this circuitry (eg pre- and postsynaptic mechanisms) could theoretically have opposite and thereby neutralizing effects on basal circuit function, but might be manifested in differential stimulated behavioral responses to APO (eg greater motor suppression) *vs* AMPH (eg greater locomotor activation). We now report that heritable strain differences in DA-stimulated [^{35}S]GTPγS binding have a strong negative correlation to PPI DA agonist sensitivity. While both D_2-like receptor-coupled G-protein function and PPI DA sensitivity appear to be influenced by genes that distinguish SD and LE strains, it is possible that these processes are responding to opposing sides of a forebrain DA regulatory feedback mechanism. In contrast, parallel locomotor- and functional receptor effects of DA stimulation may suggest that these two patterns reflect shared mechanisms in this regulatory

Table 3 Associations with a Heritable PPI Phenotype

Greater sensitivity to PPI-disruptive effects of DA agonists in SD vs LE rats IS associated with

1. Lower basal levels of BG DA turnover (Swerdlow *et al*, 2005).
2. Lower levels of DA-stimulated [^{35}S]GTPγS binding (present study).[a]
3. Lower sensitivity to the locomotor-activating effects of DA agonists (present study).[a]
4. Higher sensitivity to the motor-suppressant effects of APO (present study).[a]
5. Relatively less fur pigmentation (present study).[b]

Greater sensitivity to PPI-disruptive effects of DA agonists in SD vs LE rats IS NOT associated with

1. Differential DA agonist-stimulated changes in forebrain DA or 5HT activity (Swerdlow *et al*, 2005).
2. Different levels of basal [^{35}S]GTPγS binding (present study).
3. Differential sensitivity to DA agonist effects on startle magnitude (Swerdlow *et al*, 2002, 2003, 2004a, b; present study).[a]
4. Differential sensitivity to the PPI-disruptive effects of NMDA antagonists or 5HT agonists (Swerdlow *et al*, 2004c).[a]

[a]SD, LE, and F1 rats.
[b]LE and F1 rats.

circuitry. The present study makes it clear that genetic differences between SD and LE strains can yield opposite patterns of DA-related phenotypes across behavioral and neurochemical measures. Presumably, the growing list of DA-linked phenotypes among SD, LE, and F1 rats (Table 3) will allow us to pinpoint substrates by which genes regulate forebrain DA function. That such genes have a powerful impact on the regulation of sensorimotor gating suggests that they might contribute to heritable deficits in sensorimotor gating observed in neuropsychiatric disorders, such as schizophrenia and Tourette Syndrome.

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