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Probing the molecular basis for an inherited sensitivity to the startle-gating disruptive effects of apomorphine in rats

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Abstract

Background—Prepulse inhibition of acoustic startle (PPI) is deficient in several heritable brain disorders. In rats, the dopamine agonist, apomorphine (APO), reduces PPI and expression of the early gene, *c-fos*, within the nucleus accumbens (NAC) core. Both of these effects are greater in Sprague–Dawley (SD) vs. Long Evans (LE) rats, and this PPI strain pattern is inherited. Here, we examined phosphorylation of cyclic-AMP response element-binding protein (CREB), a putative intermediary step between dopamine receptor stimulation and Fos expression, in SD and LE rats.

Methods—The effects of APO (vehicle vs. 0.5 mg/kg) on PPI were tested in SD and LE rats in a within-subject design. Seven days later, under conditions mimicking PPI testing, half of the rats from each strain received either vehicle or APO (0.5 mg/kg) 20 min before euthanasia. NAC CREB and phospho-CREB levels were quantified from tissue sections reacted immunohistochemically.

Results—APO reduced PPI in both strains, with a significantly greater effect in SD vs. LE rats. APO also significantly reduced NAC core phospho-CREB levels in both strains, with a significantly greater effect in SD vs. LE rats. Among SD rats receiving APO, the reduction in NAC core CREB phosphorylation correlated significantly with the APO-induced reduction in PPI ($R=0.49$).

Conclusions—A dose of APO that disrupts PPI of acoustic startle causes a profound suppression of CREB phosphorylation in the NAC; both dopamine-sensitive behavioral and molecular phenotypes are more robust in SD vs. LE rats, and within SD rats, they are significantly correlated.

Keywords

Apomorphine; cAMP response element-binding protein; Dopamine; Nucleus accumbens; Prepulse inhibition; Schizophrenia; Sensorimotor gating; Startle; Strain

Introduction

Prepulse inhibition (PPI) is the diminution of the startle reflex when the startling stimulus is preceded by a weak prepulse (Graham 1975). PPI is used as an operational measure of sensorimotor gating and is deficient in certain brain disorders, including schizophrenia (Braff et al. 1978; cf. Swerdlow et al. 2008). While PPI is mediated within the pons (cf.

Koch and Schnitzler 1997), it is regulated by interconnected forebrain circuitry, including portions of the limbic cortex, striatum, pallidum, and thalamus (cf. Swerdlow et al. 2001a).

In rats, PPI is reduced by dopamine agonists, like the mixed D1/D2 receptor agonist, apomorphine (APO; Swerdlow et al. 1986, cf. 2008). PPI is under strong genetic control in rodents (Francis et al. 2003) and humans (Greenwood et al. 2007), and the neurochemical regulation of PPI also appears to be a heritable phenotype (Swerdlow et al. 2004a, b). For example, albino Sprague–Dawley (SD) rats are more sensitive to the PPI-disruptive effects of APO compared to hooded Long Evans (LE) rats (Swerdlow et al. 2001b); these differences are inherited with simple generational patterns (Swerdlow et al. 2004a, b), are present at the earliest measurable age (Swerdlow et al. 2004b), are neurochemically specific (Swerdlow et al. 2003a, 2004c), and are not due to differences in maternal behavior (Swerdlow et al. 2004b). Strain differences in PPI APO sensitivity are accompanied by differences in nucleus accumbens (NAC) gene expression (Shilling et al. 2008) and D2-mediated G-protein coupling (Swerdlow et al. 2006), which culminates downstream with differences in sensitivity to NAC APO-suppressed c-Fos expression (Saint Marie et al. 2006). One rationale for characterizing these genetically divergent NAC signal transduction pathways is to identify potential sites for more targeted therapeutics in disorders of deficient PPI, such as schizophrenia.

Because D2 G-protein coupled receptors are known to inhibit adenylate cyclase activity and, hence, cAMP response element-binding protein (CREB) phosphorylation (Gilman 1987; cf. Nestler 2001), we hypothesized that (1) PPI-disruptive effects of APO should be accompanied by suppression of NAC phospho-CREB production; (2) this inhibition of NAC phospho-CREB production should be greater in SD than LE rats; and (3) within individual rats, relative sensitivity to this inhibition should correlate significantly with sensitivity to the PPI-disruptive effects of APO. To test these hypotheses, we assessed the PPI-disruptive and NAC phospho-CREB-inhibiting effects of APO in the same groups of SD and LE rats; the magnitude of the behavioral and signal transduction effects of APO was then compared between strains and within individual animals.

Methods

Adult male SD ($n=44$) and LE ($n=44$) rats (225–249 g, Harlan Laboratories; Livermore, CA, USA) were used in this study; the final sample of $n=40$ and 42, respectively, reflects a total of ten slices of brain tissue lost due to folding during processing (<2%, see below). They were handled within 3 days of their arrival, housed in groups of two to three with unrestricted food and water, and maintained on a reversed 12/12-h light/dark cycle (lights on at 1900 to 0700 hours) for at least 1 week for acclimation before behavioral testing. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by the UCSD Animal Subjects Committee (protocol number S01221).

Behavioral testing

Startle chambers (SR-LAB Startle Reflex System; San Diego Instruments) were housed within 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 (0–4,095), 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. Testing occurred during the dark phase. Rats 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 trials (40 ms–120 dB (A) noise bursts) that were interspersed with three PP12dB+P-ALONE trials (P-ALONE preceded 100 ms (onset-to-onset) by a 20-ms noise burst of 12 dB above background).

Testing continued 4 days later. Rats were assigned to dose order groups based on average %PPI from the matching session to ensure similar baseline PPI levels between groups. All animals received either APO (0.5 mg/kg, s.c.) or vehicle (0.01% 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 7 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. This APO dose and test design were chosen because they are known to result in robust SD>LE sensitivity to the PPI-disruptive effects of APO (Swerdlow et al. 2004b).

Tissue processing and pCREB-CREB immunohistochemistry

Five to seven days later, rats were brought to the laboratory where they were housed individually and acclimated for 2+h and then injected with either vehicle or APO (0.5 mg/kg, s.c.), with dose and strain groups balanced for temporal proximity of last APO exposure (i.e., whether APO 0.5 mg/kg was administered during week 1 or week 2 of behavioral testing). They were then immediately placed in a startle chamber with a 70 dB(A) background white noise for 20 min to simulate the conditions of the PPI test, but without the startling stimuli. Once removed from the startle chamber, each rat was given a lethal overdose of pentobarbital and perfused while under deep anesthesia. The perfusion consisted of a brief (30 s) washout with ice-cold phosphate-buffered (pH 7.4) saline (± 50 mM NaF) followed by 2.5 min of ice-cold phosphate-buffered 4% paraformaldehyde (± 50 mM NaF). Dissected brains remained chilled (4°C) in the final perfusate for 24 h and then were transferred to a cryoprotective solution containing 30% sucrose in perfusate for 3 days at 4°C. Brains were frozen-sectioned in the transverse plane at 40 μ m and collected in Tris-buffered (pH 7.5) saline+50 mM NaF (TBS+NaF). Paired adjacent sections at ~240 μ m intervals containing the NAC were incubated overnight with either a rabbit antibody to pCREB (phosphorylated at serine 133, Cell Signaling Technology; Danvers, MA, USA) diluted 1:300 or with a rabbit antibody to total CREB (Cell Signaling Technology) diluted

1:2,000. Antibody binding was visualized via standard procedures using a biotinylated anti-rabbit IgG secondary antibody (Jackson ImmunoResearch; West Grove, PA, USA) and a Vectastain Elite ABC kit with a Ni-enhanced DAB reaction (Vector Laboratories; Burlingame, CA, USA). All reagents and washes for the pCREB immunohistochemistry up to the Vectastain incubation were performed with TBS+NaF to minimize de-phosphorylation during tissue processing. Rats were processed in three separate experimental groups of 24 or 32, balanced for strain, APO exposure for PPI testing (week 1 or week 2), and drug for pCREB expression. Sections exhibited no positive staining on neuronal nuclei if either the pCREB or CREB antibodies were eliminated from the processing or if either the secondary or ABC antibodies were eliminated. Additional antibody specificity tests are provided in the Vendors' product sheets.

Quantitative image analysis

Regions of the NAC were sampled at three AP levels (Fig. 2) and digitized with a Leitz Laborlux S microscope, $\times 20$ objective, and Polaroid DMC1 digital camera. These three AP levels included two that sampled exclusively from the NAC core (+1.6 and +2.2) and one that sampled exclusively from the rostral NAC shell (+2.7). Images (1,200 $\times$ 1,600 pixels, equivalent to 380 $\times$ 505 μ m of tissue section) were captured blind to strain or experimental condition. At the beginning of each session, illumination was set to maximize the gray level range without saturation. For each session, an image from a blank slide was taken to correct sample images captured during that session for non-uniformities in background illumination. Subsequent processing was automated to avoid investigator bias. Local contrast was enhanced with a batch file in Adobe PhotoShop using an unsharp mask (amount, 250%; threshold, 64 gray levels; radius, 25 pixels; as per Saint Marie et al. 2006), and anti-pCREB and anti-CREB labeled nuclei were identified and counted with a macro-file constructed in Image-Pro Plus (ver. 4.0, Media Cybernetics; Bethesda, MD, USA) that automatically thresholded objects based on gray level (0–127) and size (area $\geq$ 100 pixels).

The degree of labeling was expressed as the total area occupied by objects in the captured field that met these criteria. Extraneously identified objects, such as dirt, were eliminated from the count by investigator intervention, which occurred rarely. Parameters were maximized for each of the antibodies (pCREB and CREB) so that positively stained nuclei were dark against a light background. In this way, we could compare the percent area occupied by positively stained nuclei in the captured image field. This was necessary rather than merely counting individually stained nuclei as we have done with cFOS-positive nuclear staining (Saint Marie et al. 2006) because the large numbers of positively-stained nuclei in the 40- μ m thick sections often caused overlying nuclei to appear as single larger irregularly shaped objects (especially with CREB staining) in the flattened two-dimensional images. We chose instead to compare the percent area of the image field occupied by pCREB stained nuclei with the percent area occupied by CREB-stained nuclei in an immediately adjacent tissue section.

In a small number of tissue sections, folds led to opaque hemispheres and the loss of meaningful data. This loss of data from a single hemisphere at a single AP level voided all molecular and behavioral data from a given rat based on the structure of both the repeated-

measure ANOVA of the molecular data, and the regression of behavioral and molecular data. For this reason, where possible, values from the opposite hemisphere at the same AP level were used as surrogates; this was not possible in six cases ($n=4$ SD, $n=2$ LE), which were thus eliminated from analyses. Analyses with, and without, this “recovery” process yielded comparable results.

Statistical analysis

PPI was defined as $100 - ((\text{startle amplitude on prepulse trials} / \text{startle amplitude on PULSE trials}) \times 100)$ 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. A measure of drug “effect” (“%APO suppression” = mean PPI after vehicle minus mean PPI after APO) was 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. 2004b, 2006).

In order to compare the suppressive effects of systemic APO on CREB phosphorylation (a between-subject analysis) and PPI (a within-subject analysis) in the same animals, the % APO suppression of pCREB was assessed relative to a normalized “vehicle” level (mean pCREB/CREB percent area) for each strain, AP level, side, and iteration of the experiment. The %APO suppression of CREB phosphorylation for each APO-treated rat was then calculated by subtracting the post-APO value from the appropriate mean vehicle-treated value, and dividing by that same mean vehicle value, i.e., $(100 \times (\text{mean vehicle value} - \text{APO value}) / \text{mean vehicle value})$. %APO suppression for all APO-treated rats was then analyzed by ANOVA, with strain as a between-subject factor, and bregma level and side as within-subject values. Simple regression analyses were used to assess the relationship between %APO suppression of PPI and CREB phosphorylation within APO-treated rats. Alpha for all simple main comparisons was 0.05.

Results

Behavior

As previously reported with prepulse intervals in the range of 100 ms (e.g., Swerdlow et al. 2001b), compared to LE rats, SD rats were significantly more sensitive to the PPI-disruptive effects of APO (Fig. 1a). ANOVA of PPI revealed significant effects of strain ($F=21.83$; $df=1, 80$; $p<0.0001$) and APO dose ($F=257.20$; $df=1, 80$; $p<0.0001$), and a significant interaction of strain×APO dose ($F=56.65$; $df=1, 80$; $p<0.0001$). There was a significant effect of prepulse intensity ($F=93.02$; $df=2, 160$; $p<0.0001$) and a significant three-way interaction of strain×APO dose×intensity ($F=7.62$; $df=2, 160$; $p<0.001$). Post-hoc comparisons confirmed significantly greater APO-induced disruption of PPI in SD vs. LE rats at each of the three prepulse intensities.

ANOVA of startle magnitude on pulse-alone trials also detected a less-consistent finding in previous reports: compared to SD rats, LE rats were significantly more sensitive to the startle-enhancing effects of APO (Fig. 1b). ANOVA of startle magnitude during PPI testing confirmed a significant effect of strain ($F=14.53$; $df=1, 80$; $p<0.0005$) and APO dose ($F=37.32$; $df=1, 80$; $p<0.0001$) and a significant interaction of strain×APO dose ($F=22.36$; $df=1, 80$; $p<0.0001$). Post-hoc comparisons confirmed that APO potentiated startle magnitude in LE rats ($F=45.26$; $df=1, 41$; $p<0.0001$) but not in SD rats ($F=1.43$; $df=1, 39$; ns).

To assess the potential impact of this differential strain APO-sensitivity of *startle magnitude* on that observed for *PPI*, median splits were used to divide SD and LE groups into those exhibiting the most vs. least APO-potentiated startle magnitude. ANOVA of PPI using this “startle sensitivity” factor confirmed significant main effects of strain and APO dose, and the strain×APO dose interaction (all p 's<0.0001), but detected no significant interaction of “startle sensitivity” with any of these factors (e.g., the critical three-way interaction of strain×APO dose×startle sensitivity: $F<0.1$). A number of other agnostic analytic strategies (see Fig. 1c, d) confirmed that the strain differences in PPI were independent of those on startle magnitude; for example, when APO effects on startle magnitude were balanced across strains by eliminating quartiles of “least startle-sensitive” SD rats and “most startle-sensitive” LE rats, ANOVA of PPI confirmed all significant findings (e.g., strain×APO dose, $p<0.0001$).

NAC CREB phosphorylation

The suppressive effect of APO on percent area occupied by pCREB-stained nuclei relative to that of CREB-stained nuclei are illustrated graphically for both rat strains in Fig. 2.

Examples of captured image fields illustrating nuclear staining with the pCREB and CREB antibodies after systemic injection with APO (vehicle vs. 0.5 mg/kg, s.c.) are shown in Fig. 3. These images represent the mean level of staining under each set of conditions (examples are from SD rats at AP +2.20). Raw CREB and pCREB values are found in Table 1.

ANOVAs confirmed that pCREB levels were significantly reduced by APO in the NACc ($F=10.59$; $df=1, 76$; $p<0.002$), due primarily to its effects in SD rats ($F=11.07$; $df=1, 37$; $p=0.002$) compared to LE rats ($F=1.38$; $df=1, 39$; ns). Similarly, in the rostral NAC shell, APO significantly suppressed pCREB ($F=10.60$; $df=1, 72$; $p<0.002$); this effect was robust in SD rats ($F=9.21$; $df=1, 35$; $p<0.005$), but narrowly reached statistical significance in LE rats ($F=3.99$; $df=1, 37$; $p=0.053$).

The %APO suppression of CREB phosphorylation for the three AP levels are illustrated in Fig. 2c. ANOVA of % APO suppression in the NACc revealed a significant main effect of strain ($F=5.05$; $df=1, 40$; $p<0.05$) reflecting great APO-induced suppression of CREB phosphorylation in SD vs. LE rats. This difference was not evident in the rostral shell ($F<1$).

Regression analysis was then used to assess the relationship between the two “APO effects” within rats: suppression of PPI and suppression of CREB phosphorylation (based on the mean values of pCREB/CREB (percent area) from the two sides and two NAC core brain levels (+2.2 and +1.6) for each rat that received APO on the “terminal” day of the

experiment). Analyses revealed that these effects of APO were moderately but significantly correlated ($R=0.371$, $p<0.05$), based on a significant relationship among SD rats ($R=0.49$, $p<0.05$) but not LE rats ($R=-0.06$, ns; Fig. 4). This correlation was significantly different between strains (Fisher r -to- z transformation; $p<0.01$). Thus, APO suppressed PPI (predominantly in SD rats) and CREB phosphorylation (predominantly in SD rats), and these two effects were significantly and positively correlated within individual SD but not LE rats. In contrast, a weaker and non-significant relationship between APO effects on PPI and CREB phosphorylation was detected in the rostral NAC shell (overall R for APO effects on PPI and CREB phosphorylation: $R=0.20$, ns; SD $R=0.36$, ns; LE $R=0.15$, ns). APO effects on NAC core CREB phosphorylation were not significantly related to the magnitude of APO effects on startle magnitude on pulse-alone trials (NACc: $R=-0.12$, ns; NAC shell: $R=-0.04$), and this was true in both SD and LE rats (NACc: R 's=0.16 and 0.03, respectively; NAC shell R 's=0.17 and -0.04 , respectively).

Discussion

PPI is a simple operational measure of sensorimotor gating; while there is no clear understanding of what PPI “means” in terms of real-life function, its utility as an experimental model largely reflects two facts: (1) it lends itself to detailed cross-species neurobiological analyses, at levels ranging from systems (Qu et al. 2009; Kumari et al. 2005) to cells (Saint Marie et al. 2006) to molecules (e.g., Culm et al. 2003; Swerdlow et al. 2006) to genes (Shilling et al. 2008; Greenwood et al. 2007); and (2) it is deficient in several neuropsychiatric disorders (cf. Braff et al. 2001; Swerdlow et al. 2008). Many interventions in rodents can model PPI deficits observed in human brain disorders, and in some cases, these interventions are linked biologically to constructs associated with those disorders. For example, the link between dopamine (DA) dysregulation and disorders like schizophrenia and Tourette syndrome (TS)—both characterized by deficient PPI (Braff et al. 1978; Castellanos et al. 1996)—makes the disruption of PPI by DA agonists a potentially informative laboratory model. Further support for the potential utility of this model comes from evidence that the PPI-disruptive effects of APO (1) are exaggerated by developmental manipulations associated with schizophrenia (Lipska et al. 1995); (2) are opposed by antipsychotics in a manner that significantly predicts ($R=0.99$) their clinical potency (Swerdlow et al. 1994); and (3) differ in potency across strains, in a manner that is inherited, and strongly related to the region-specific expression of certain genes linked to schizophrenia and other disorders (e.g., COMT, $p<10^{-17}$ (Shilling et al. 2008)). Conceivably, by understanding the molecular processes responsible for an inherited phenotype of PPI “DA-disruptability,” we might identify biological targets that explicate the disruption of PPI in disorders such as schizophrenia and TS, and that serve as targets for clinical remediation.

A focus on the nucleus accumbens as a region critical to the regulation of PPI came initially from our observations that the startle-inhibiting effects of pulsating tactile stimuli were disrupted by ablative lesions of the NAC (Sorenson and Swerdlow 1982), and that 6-hydroxydopamine denervation of the NAC resulted in a “supersensitivity” to the PPI-disruptive effects of the direct DA agonist, APO (Swerdlow et al. 1986), and a diminution of the PPI-disruptive effects of the indirect DA agonist, amphetamine (Swerdlow et al. 1990).

Subsequent studies confirmed that PPI was disrupted by direct infusion of DA (Swerdlow et al. 1990) or D2-like agonists into the NAC, and predominantly into the NAC core subregion (Wan et al. 1994). This line of inquiry eventually led to the description of an interconnected forebrain PPI-regulatory circuit and its descending influences on a pontine PPI-mediating circuit (cf. Swerdlow et al. 2001a; Koch and Schnitzler 1997). Evidence now suggests that sensitivity to the DAergic regulation of PPI in rats is an inherited phenotype linked to processes at the “input” to NAC medium spiny neurons (MSNs; e.g., genes regulating DA metabolism (Shilling et al. 2008) and GTPgammaS binding (Swerdlow et al. 2006)) and processes at the “output” of NAC MSNs (e.g., NAC cFOS expression (Saint Marie et al. 2006) and ventral pallidal GABA efflux (Qu et al. 2009)). The present study examines one substrate within the NAC DA signal transduction pathway as an intermediate molecular step controlling this inherited phenotype by bridging MSN input and output.

At a dose that disrupted PPI, preferentially in SD vs. LE rats, APO reduced NAC core CREB phosphorylation, also preferentially in SD vs. LE rats. To our knowledge, this finding provides the first evidence for such inherited strain differences at this level of DA-mediated signal transduction; of course, the more relevant issue is that these differences are associated with a behavioral phenotype that is highly heritable (Greenwood et al. 2007) and deficient in DA-linked brain disorders (cf. Swerdlow et al. 2008). The premise behind animal models of this kind is that the present findings suggest a biological mechanism that, if conserved across mammalian species, might contribute to a loss of PPI—and conceivably any functional consequences associated with such a loss (Swerdlow et al. 2006)—in these human brain disorders.

The fact that the APO sensitivity of the behavioral phenotype (PPI) in SD rats is correlated significantly with the sensitivity of the molecular phenotype (NAC core CREB phosphorylation) suggests that these macro- and micro-phenomena are related, but does not demonstrate a causal link. The possibility of such a causal link is suggested by findings that “recovery” from disruption of PPI after repeated D2-(like) stimulation is accompanied by increased NAC CREB phosphorylation, and blockade of NAC CREB phosphorylation “rescues” the D2-(like)-mediated PPI disruption (Berger et al. 2011). However, any such “causal link” appears to be categorically different between SD and LE rats—based on the lack of correlation between APO effects on PPI and CREB phosphorylation in LE rats—suggesting that the differential PPI APO sensitivity across these strains is not simply a function of quantitative “sensitivity,” but may in fact reflect distinct molecular mechanisms. While the significant correlation ($R=0.49$) in SD rats suggests that either one of the two phenotypes can account for only a modest amount of the variance in the other, it is nonetheless remarkable that the reduced phosphorylation of one particular intracellular protein in one specific region of the ventral forebrain could account for any significant amount of the variance of a complex behavioral phenotype like PPI drug sensitivity. Other brain regions are known to contribute to the DAergic regulation of PPI in SD rats, and almost certainly, other signaling pathways also regulate this phenotype; thus, even a modest correlation between these phenotypes is notable. However, it is also important to comment on several potential limitations in the present study that complicate any straightforward—let alone causal—interpretation of our findings.

First, only a single dose of APO was used. This dose was selected because, after numerous dose–response studies in SD and LE rats (e.g., Swerdlow et al. 2001b; Weber and Swerdlow 2007), 0.5 mg/kg is consistently associated with SD>LE PPI sensitivity, at 100-ms prepulse intervals. Lower doses (e.g., 0.1 mg/kg) are often below the threshold needed to reliably disrupt PPI in SD rats under the present stimulus conditions (e.g., Swerdlow et al. 1986, 2001b), while higher doses (e.g., 1.0 mg/kg) can produce maximal PPI disruption (Mansbach et al. 1988), and hence “ceiling” effects that might obviate strain differences. Conceivably, strain differences in sensitivity to APO-suppressed CREB phosphorylation might have followed a different dose–response pattern (e.g., monotonic ascending) that would complicate a simple “linear” interpretation of the relationship between the behavioral and molecular phenotypes. Based on the apparent “uncoupling” of PPI and NAC pCREB sensitivity to APO in LE rats (Fig. 3), we would predict that, even if higher APO doses were to suppress NAC CREB phosphorylation in LE rats, these effects would not correlate significantly with PPI APO sensitivity. Second, only a single set of stimulus parameters was used: one prepulse interval (100 ms) and three prepulse intensities, each of which yielded the “desired” SD>LE strain difference in PPI APO sensitivity. Like the choice of APO dose, these stimulus parameters were selected out of our awareness from numerous past studies that they generate a phenotype with such a simple, reliable strain difference (Swerdlow et al. 2001b, 2004b, c). In contrast, we know (Swerdlow et al. 2004a, c; Shilling et al. 2008) that more (and different) information about this strain difference can be elicited using a range of prepulse intervals (e.g., 10–120 ms): while SD rats are *more* sensitive than LE rats to the PPI-disruptive effects of APO at *long* (60–120 ms) prepulse intervals, they are *less* sensitive than LE rats to the PPI-enhancing effects of APO at *short* (10–30 ms) prepulse intervals, and it is this temporal pattern of PPI APO sensitivity that is clearly inherited in these strains. Because of our decision to focus on one simple aspect of this complex phenotype, we have no way of knowing from the present studies whether the PPI-enhancing effects of APO at short prepulse intervals are related in any way to the observed APO effects on NAC core CREB phosphorylation. Third, analyses of the NAC shell were limited to a single, rostral level, leaving open the question of whether strain differences in APO effects on CREB phosphorylation might be detected in more caudal portions of the NAC shell. To address this question, we completed separate measurements of CREB phosphorylation in the caudal NAC shell (AP +2.2 and +1.6), matching animals and levels reported above for the NAC core. Analyses confirmed that APO suppressed CREB phosphorylation in these caudal levels of the NAC shell, and that while this effect was arithmetically greater in SD vs. LE rats, the magnitude of this strain difference was substantially smaller than that detected in the NAC core ($d=0.25$ vs. 0.56 , respectively) and did not approach statistical significance ($F<1$, $p>0.3$; unpublished data).

One explicit long-term goal of the present line of inquiry is to identify “druggable” molecular targets for normalizing sensorimotor gating in specific DA-linked, inherited neuropsychiatric disorders (Wang and Insel 2010). In this regard, it is particularly important to note that reduced NACc CREB phosphorylation appears to be linked to a DA-mediated loss of sensorimotor gating only in one of two rat strains. Clearly, if the role of this signaling process in regulating PPI is not shared by two different rat strains, we cannot be confident that it will be an important substrate regulating DA-linked gating deficits in humans; we are

unaware of whether such strain differences have been examined in other common cross-species models of DAergic effects on NAC CREB phosphorylation (e.g., Carlezon et al. 1998). DA agonists can reduce, or increase, PPI in healthy humans, depending on the drug, and the genotype or genotype-suggestive traits of the individual (Swerdlow et al. 2003b; Talledo et al. 2009; Bitsios et al. 2005; Roussos et al. 2008). Conceivably, among humans most sensitive to the PPI-disruptive effects of some DA agonists, there might be a relationship between this sensitivity and reduced NAC CREB phosphorylation, analogous to that observed in SD rats in the present study. For example, such individuals exhibit traits suggestive of the low activity Val/Val genotype of the Val158Met COMT polymorphism (e.g., Golimbet et al. 2007; Roussos et al. 2008), and SD rats exhibit levels of NAC COMT gene expression that are significantly lower than those in LE rats (Shilling et al. 2008). Even so, the present findings suggest that the apparent relationship between PPI DA sensitivity and NAC CREB phosphorylation is variable across strains, and hence, we cannot assume that this signaling mechanism would be a suitable target for regulating sensorimotor gating in human clinical populations.

Perhaps the most parsimonious explanation for the lack of correlation between the behavioral and molecular phenotypes in LE rats is that, based on the apparent reduced impact of NAC DA D2 receptor stimulation on PPI in LE rats, DAergic activity in other brain regions and/or on other DA receptors provides a dominant influence on PPI. For example, in LE rats, basolateral amygdala (BLA) DAergic tone is known to potently regulate PPI: PPI is enhanced by intra-BLA infusion of D1 antagonists and is disrupted by intra-BLA infusion of D2 antagonists (Stevenson and Gratton 2004). Conceivably, the effects of APO on BLA or other D1 receptors might dominate the behavioral “signal” in this strain, and thereby obviate any correlation with NAC DA D2 signaling; a dominant influence of APO on D1 mechanisms in LE rats might also contribute to the robust startle-enhancing effects of APO, which are thought to be mediated substantially via D1 receptor stimulation (Schwarzkopf et al. 1993; Meloni and Davis 1999).

In summary, SD vs. LE differences in sensitivity to the PPI-disruptive effects of the DA agonist APO parallel differences in sensitivity to the APO-induced suppression of NAC CREB phosphorylation. The relationship between the APO sensitivities of PPI and NAC p-CREB also differs across strains, with PPI APO sensitivity being significantly correlated with p-CREB APO sensitivity in SD but not LE rats. To the degree that extrapolation to humans is appropriate, one implication of these “strain-specific” findings is that suppression of NAC CREB phosphorylation might be a target for therapeutic interventions only for certain clinical subgroups, in whom these behavioral and molecular phenotypes are causally linked.

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References

- Berger AK, Green T, Siegel SJ, Nestler EJ, Hammer RP Jr. cAMP response element binding protein phosphorylation in nucleus accumbens underlies sustained recovery of sensorimotor gating following repeated D(2)-like receptor agonist treatment in rats. *Biol Psychiatry*. 2011; 69:288–294. [PubMed: 21035786]
- Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L. Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology*. 1978; 14:339–343.
- Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology*. 2001; 156:234–258. [PubMed: 11549226]
- Bitsios P, Giakoumaki SG, Frangou S. The effects of dopamine agonists on prepulse inhibition in healthy men depend on baseline PPI values. *Psychopharmacology*. 2005; 182:144–152. [PubMed: 16010540]
- Carlezon WA Jr, Thome J, Olson VG, Lane-Ladd SB, Brodtkin ES, Hiroi N, Duman RS, Neve RL, Nestler EJ. Regulation of cocaine reward by CREB. *Science*. 1998; 282:2272–2275. [PubMed: 9856954]
- Castellanos FX, Fine EJ, Kaysen D, Marsh WL, Rapoport JL, Hallett M. Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. *Biol Psychiatry*. 1996; 39:33–41. [PubMed: 8719124]
- Culm KE, Lim AM, Onton JA, Hammer RP Jr. Reduced Gi and Go protein function in the rat nucleus accumbens attenuates sensorimotor gating deficits. *Brain Res*. 2003; 982:12–18. [PubMed: 12915235]
- Francis DD, Szegea K, Campbell G, Martin WD, Insel TR. Epigenetic sources of behavioral differences in mice. *Nat Neurosci*. 2003; 6:445–446. [PubMed: 12665797]
- Gilman AG. G proteins: transducers of receptor-generated signals. *Annu Rev Biochem*. 1987; 56:615–649. [PubMed: 3113327]
- Golimbet VE, Alfimova MV, Gritsenko IK, Ebstein RP. Relationship between dopamine system genes and extraversion and novelty seeking. *Neurosci Behav Physiol*. 2007; 37:601–606. [PubMed: 17657431]
- Graham F. The more or less startling effects of weak prestimuli. *Psychophysiology*. 1975; 12:238–248. [PubMed: 1153628]
- Greenwood TA, Braff DL, Light GA, Cadenhead KS, Calkins ME, Dobie DJ, Freedman R, Green MF, Gur RE, Gur RC, Mintz J, Nuechterlein KH, Olincy A, Radant AD, Seidman LJ, Siever LJ, Silverman JM, Stone WS, Swerdlow NR, Tsuang DW, Tsuang MT, Turetsky BI, Schork NJ. Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. *Arch Gen Psychiatry*. 2007; 64:1242–1250. [PubMed: 17984393]
- Koch M, Schnitzler HU. The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. *Behav Brain Res*. 1997; 89:35–49. [PubMed: 9475613]
- Kumari V, Antonova E, Zachariah E, Galea A, Aasen I, Ettinger U, Mitterschiffthaler MT, Sharma T. Structural brain correlates of prepulse inhibition of the acoustic startle response in healthy humans. *Neuroimage*. 2005; 26:1052–1058. [PubMed: 15961045]
- Lipska BK, Swerdlow NR, Geyer MA, Jaskiw GE, Braff DL, Weinberger DR. Neonatal excitotoxic hippocampal damage in rats causes post-pubertal changes in prepulse inhibition of startle and its disruption by apomorphine. *Psychopharmacology*. 1995; 122:35–43. [PubMed: 8711062]
- Mansbach RS, Geyer MA, Braff DL. Dopaminergic stimulation disrupts sensorimotor gating in the rat. *Psychopharmacology*. 1988; 94:507–514. [PubMed: 3131796]
- Meloni EG, Davis M. Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. *Psychopharmacology*. 1999; 144:373–380. [PubMed: 10435410]
- Nestler EJ. Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci*. 2001; 2:119–128. [PubMed: 11252991]
- Paxinos, G., Watson, C. The rat brain in stereotaxic coordinates. 4. Academic; San Diego: 1998.
- Qu Y, Saint Marie RL, Breier MR, Ko D, Stouffer D, Parsons LH, Swerdlow NR. Neural basis for a heritable phenotype: differences in the effects of apomorphine on startle gating and ventral pallidal

- GABA efflux in male Sprague-Dawley and Long-Evans rats. *Psychopharmacology*. 2009; 207:271–280. [PubMed: 19756524]
- Roussos P, Giakoumaki SG, Rogdaki M, Pavlakis S, Frangou S, Bitsios P. Prepulse inhibition of the startle reflex depends on the catechol *O*-methyltransferase Val158Met gene polymorphism. *Psychol Med*. 2008; 38:1651–1658. [PubMed: 18261249]
- Saint Marie RL, Neary AC, Shoemaker JM, Swerdlow NR. The effects of apomorphine and D-amphetamine on striatal c-Fos expression in Sprague-Dawley and Long Evans rats and their F1 progeny. *Brain Res*. 2006; 1119:203–214. [PubMed: 16979142]
- Schwarzkopf SB, Bruno JP, Mitra T. Effects of haloperidol and SCH 23390 on acoustic startle and prepulse inhibition under basal and stimulated conditions. *Prog Neuropsychopharmacol Biol Psychiatry*. 1993; 17:1023–1036. [PubMed: 8278596]
- Shilling PD, Saint Marie RL, Shoemaker JM, Swerdlow NR. Strain differences in the gating-disruptive effects of apomorphine: relationship to gene expression in nucleus accumbens signaling pathways. *Biol Psychiatry*. 2008; 63:748–758. [PubMed: 18083141]
- Sorenson CA, Swerdlow NR. The effects of tail pinch on the acoustic startle response in rats. *Brain Res*. 1982; 247:105–113. [PubMed: 7127106]
- Stevenson CW, Gratton A. Role of basolateral amygdala dopamine in modulating prepulse inhibition and latent inhibition in the rat. *Psychopharmacology*. 2004; 176:139–145. [PubMed: 15114433]
- Swerdlow NR, Geyer M, Braff D, Koob GF. Central dopamine hyperactivity in rats mimics abnormal acoustic startle in schizophrenics. *Biol Psychiatry*. 1986; 21:23–33. [PubMed: 3080033]
- Swerdlow NR, Mansbach RS, Geyer MA, Pulvirenti L, Koob GF, Braff DL. Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. *Psychopharmacology*. 1990; 100:413–416. [PubMed: 2315438]
- Swerdlow NR, Braff DL, Taaid N, Geyer MA. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Arch Gen Psychiatry*. 1994; 51:139–154. [PubMed: 8297213]
- Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology*. 2001a; 156:194–215. [PubMed: 11549223]
- Swerdlow NR, Platten A, Kim YK, Gaudet I, Shoemaker J, Pitcher L, Auerbach P. Sensitivity to the dopaminergic regulation of prepulse inhibition in rats: evidence for genetic, but not environmental determinants. *Pharmacol Biochem Behav*. 2001b; 70:219–226. [PubMed: 11701191]
- Swerdlow NR, Shoemaker JM, Platten A, Pitcher L, Goins J, Crain S. Heritable differences in the effects of amphetamine but not DOI on startle gating in albino and hooded outbred rat strains. *Pharmacol Biochem Behav*. 2003a; 75:191–197. [PubMed: 12759127]
- Swerdlow NR, Stephany N, Wasserman LC, Talledo J, Shoemaker J, Auerbach PP. Amphetamine effects on prepulse inhibition across species: replication and parametric extension. *Neuropsychopharmacology*. 2003b; 28:640–650. [PubMed: 12655308]
- Swerdlow NR, Shoemaker JM, Auerbach PP, Pitcher L, Goins J, Platten A. Heritable differences in the dopaminergic regulation of sensorimotor gating: II. Temporal, pharmacologic and generational analyses of apomorphine effects on prepulse inhibition. *Psychopharmacology*. 2004a; 174:452–462. [PubMed: 15300359]
- Swerdlow NR, Shoemaker JM, Crain S, Goins J, Onozuka K, Auerbach PP. Sensitivity to drug effects on prepulse inhibition in inbred and outbred rat strains. *Pharmacol Biochem Behav*. 2004b; 77:291–302. [PubMed: 14751457]
- Swerdlow NR, Shoemaker JM, Platten A, Pitcher L, Goins J, Auerbach PP. Heritable differences in the dopaminergic regulation of sensorimotor gating: I. Apomorphine effects on startle gating in albino and hooded outbred rat strains and their F1 and N2 progeny. *Psychopharmacology*. 2004c; 174:441–451. [PubMed: 15300358]
- Swerdlow NR, Krupin AS, Bongiovanni MJ, Shoemaker JM, Goins JC, Hammer RP Jr. Heritable differences in the dopaminergic regulation of behavior in rats: relationship to D2-like receptor G-protein function. *Neuropsychopharmacology*. 2006; 31:721–729. [PubMed: 16123742]

- Swerdlow NR, Weber M, Qu Y, Light GA, Braff DL. Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology*. 2008; 199:331–388. [PubMed: 18568339]
- Talledo J, Sutherland Owens A, Schortenghuis T, Swerdlow NR. Amphetamine effects on startle gating in normal women and female rats. *Psychopharmacology*. 2009; 204:165–175. [PubMed: 19148623]
- Wan FJ, Geyer MA, Swerdlow NR. Accumbens D2 modulation of sensorimotor gating in rats: assessing anatomical localization. *Pharmacol Biochem Behav*. 1994; 49:155–163. [PubMed: 7816867]
- Wang PS, Insel TR. NIMH-funded pragmatic trials: moving on. *Neuropsychopharmacology*. 2010; 35:2489–2490. [PubMed: 21068745]
- Weber MA, Swerdlow NR. Rat strain differences in startle gating-disruptive effects of apomorphine occur with both acoustic and visual prepulses. *Pharmacol Biochem Behav*. 2007; 88:306–311. [PubMed: 17900675]

Fig. 1.

Effects of APO (0.5 mg/kg, s.c.) on PPI (**a**) and startle magnitude (**b**) in SD and LE rats (n 's=40 and 42, respectively). **a** Compared to LE rats, SD rats were significantly more sensitive to the PPI-disruptive effects of APO. *Asterisks*, significantly less PPI in SD than LE after APO, $p<0.0001$. SD=open bars; LE=black bars. **b** Startle magnitude on trials with no prepulse (-) and those with prepulses of 5, 10, and 15 dB over background. Startle magnitude on pulse alone (-) trials was significantly increased in LE but not SD rats (*asterisk*, $p<0.0001$). Note that, after APO treatment, prepulses reduce startle magnitude in LE but not SD rats. **c** and **d** Subgroups of SD and LE rats in which APO had no mean impact on startle magnitude (see "-" trials, **d**) demonstrated identical pattern of SD>LE APO PPI sensitivity (**c**), and intact ability of prepulses to reduce startle magnitude after APO in LE but not SD rats (**d**). Vehicle=open circles; APO=black circles

Fig. 2.

Effects of APO on CREB phosphorylation in the NAC in SD and LE rats. **a** Illustrations showing three bregma levels in coronal sections of the rat brain from which tissue was sampled (modified from Paxinos and Watson 1998). *Gray rectangles* represent areas of the NAC shell (NACs) and core (NACc) that were sampled. **b** NAC pCREB as a percent of total CREB (see “Methods” for calculations). ANOVA revealed significant APO-suppression of CREB phosphorylation in both strains and brain regions (NAC core and rostral shell, *asterisk*). Vehicle=*open bars*; APO=*black bars*. **c** When data were analyzed to calculate the magnitude of the “APO effect” on CREB phosphorylation (relative to vehicle values in each strain/brain level/ experiment), ANOVA revealed significant SD>LE magnitude of APO effects on CREB phosphorylation in the NACc (*asterisks*). SD=*open bars*; LE=*black bars*. *N*s=40 and 42, for SD and LE, respectively

Fig. 3. Photomicrographs showing levels of staining sampled from bregma level +2.2 in A from SD and LE rats. Each photomicrograph was chosen to represent the mean level of staining for its drug×antibody group. APO visibly reduced phospho-CREB staining but not CREB staining

Fig. 4.

Simple regression of magnitude of APO effect on PPI (x -axis) and CREB phosphorylation in the NACc (y -axis) in SD (**a**, $R=0.49$, $p<0.05$) and LE (**b**, $R=-0.06$, ns) rats. These two correlations were significantly different from each other ($p<0.01$)

Table 1

Raw pCREB and CREB product at three AP levels of the NAC (mean number of pixels)

Strain	Drug	Antibody	AP +1.6	AP +2.2	AP +2.7
SD	Vehicle	pCREB	142,274	133,665	110,916
SD	APO	pCREB	95,298	71,519	63,017
LE	Vehicle	pCREB	127,544	113,634	99,040
LE	APO	pCREB	105,667	80,154	70,522
SD	Vehicle	CREB	262,770	278,025	258,795
SD	APO	CREB	281,483	278,107	273,274
LE	Vehicle	CREB	278,883	276,884	248,241
LE	APO	CREB	271,567	297,190	296,002

Raw mean values here are averaged across right and left hemispheres and are not normalized for vehicle levels or iteration of experiment; see "Methods" section