Published in final edited form as:
Biol Psychiatry. 2008 April 15; 63(8): 748–758. doi:10.1016/j.biopsych.2007.10.015.

Strain differences in the gating-disruptive effects of apomorphine: Relationship to gene expression in nucleus accumbens signaling pathways

Paul D. Shilling, **Richard L. Saint Marie**, **Jody M. Shoemaker**, and **Neal R. Swerdlow**
Department of Psychiatry University of California, San Diego

Abstract

Background—Prepulse inhibition (PPI) of startle is a measure of sensorimotor gating that is deficient in certain psychiatric disorders, including schizophrenia. Sprague Dawley (SD) rats are more sensitive to PPI-disruptive effects of apomorphine (APO) at long inter-stimulus intervals (ISIs) (60-120 ms) and less sensitive to PPI-enhancing effects of APO at short ISIs (10-30 ms) compared to Long Evans (LE) rats.

Methods—PPI was tested in SD and LE rats after APO (0.5 mg/kg) or vehicle in a within subject design, and sacrificed 14 days later. Total RNA was extracted from the nucleus accumbens (NAC). Approximately 700 dopamine-relevant transcripts on the Affymetrix 230 2.0 microarray were analyzed.

Results—As previously reported, SD rats exhibited greater APO-induced PPI deficits at long intervals and less APO-induced PPI enhancement at short intervals compared to LE rats. One hundred and four genes exhibited significantly different NAC expression levels in these two strains. Pathway analysis revealed that many of these genes contribute to dopamine receptor signaling, synaptic long-term potentiation or inositol phosphate metabolism. The expression of some genes significantly correlated with measures of APO-induced PPI sensitivity in either SD or LE rats. The expression of select genes was validated by real time RT- PCR.

Conclusions—Differences in PPI APO sensitivity in SD vs. LE rats are robust and reproducible, and may be related to strain differences in the expression of genes that regulate signal transduction in the NAC. These genes could facilitate the identification of targets for ameliorating heritable gating deficits in brain disorders such as schizophrenia.

Keywords

schizophrenia; apomorphine; dopamine; prepulse inhibition; gene expression; DNA microarray

Introduction

Identifying neural and genetic mechanisms underlying many psychiatric disorders has proven difficult. In part, this difficulty reflects a reliance on complex, descriptive and often variable

Corresponding Author: Neal R. Swerdlow. M.D., Ph.D., University of California, San Diego, Department of Psychiatry, 9500 Gilman Drive, La Jolla, CA 92093, Phone: 619-543-6270, FAX: 619-543-2493, Email: nswerdlow@ucsd.edu.

clinical phenotypes as the fulcrum for parsing biological substrates. An alternative strategy is to study the neural and genetic bases of physiological abnormalities that accompany the clinical disorders, which may be closer to the disease genes, compared to the clinical symptoms [1-3].

Prepulse inhibition (PPI) of the acoustic startle reflex is the reduction in startle magnitude when the startle-eliciting stimulus (pulse) is preceded 30 - 500 ms by a weak stimulus (prepulse) [4]. PPI is an operational measure of sensorimotor gating that is heritable [5,6] and regulated by forebrain circuitry, including portions of limbic-associated cortex and subcortical structures (cf. [7,8]). PPI is deficient in several neuropsychiatric disorders, including schizophrenia [9-11], and PPI deficits occur in rats after administration of dopamine agonists, including the indirect dopamine agonist amphetamine and the direct dopamine agonist apomorphine (APO) [12,13].

Baseline and drug-induced changes in PPI exhibit robust differences across rat strains. For example, Sprague Dawley (SD) rats exhibit significantly greater sensitivity to the PPI-disruptive effects of both amphetamine and APO [14-23] compared to Long Evans (LE) rats. This strain difference is specific to DA agonists, as these two strains do not exhibit differential sensitivity to the PPI-disruptive effects of serotonin agonists and NMDA receptor antagonists [14,19]. This strain difference in PPI sensitivity to DA agonists is heritable [17,18], independent of fostering conditions or differences in maternal-pup interactions [17] and stable across testing and breeding facilities [15], and is first observed before postnatal day 18 [18].

Several lines of evidence link SD vs. LE differences in PPI APO sensitivity to differences in DA-stimulated signal transduction in the nucleus accumbens (NAC). Thus, SD vs. LE differences in PPI APO sensitivity are accompanied by, and often correlate significantly with, differences in NAC [^{35}S]GTPγS binding [21] and in APO-induced inhibition of NAC phosphorylation of cyclic AMP response element binding protein (CREB) [24] and FOS expression [22]. F1 (SD × LE) rats exhibit intermediate phenotypes in PPI APO sensitivity [17,18,21] and in some measures of NAC DA-stimulated signal transduction [21,22].

Strain differences in NAC GTPγS binding, FOS expression, CREB phosphorylation and PPI APO sensitivity might reflect differences in the expression of genes in SD vs. LE rats that normally contribute to the regulation of DA-linked signal transduction in the NAC. Identification of these genes could provide potential molecular targets that contribute to the dopaminergic regulation of PPI in rodents and in several heritable psychiatric disorders, including schizophrenia and Tourette Syndrome [25]. DNA microarrays provide a powerful means to investigate brain regional gene expression in rodents that exhibit differences in DA agonist sensitivity [26,27]. To identify genes that might contribute to strain differences in sensitivity to the gating-disruptive effects of DA agonists, we compared NAC mRNA expression in SD and LE rats of approximately 700 transcripts associated with DA-regulated signal transduction and mRNA expression, and genes reproducibly shown to be relevant to schizophrenia. We focused exclusively on the NAC, based on evidence that this region is critically involved in the dopaminergic regulation of PPI [8,12], and specifically, in strain differences in this regulation [23] and in activity within DA-linked signal transduction pathways [21,22,24].

Methods

Twelve SD and twelve LE male rats (229 –250 grams; Harlan Laboratories, San Diego, CA) were housed and handled as per previous reports [14-24]. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the UCSD Animal Subjects Committee (protocol #S01221).

PPI Testing

Startle chambers (SR-LAB; San Diego Instruments) were located in a sound-attenuated room with a 60 dB(A) ambient noise. A brief startle session was used to form balanced drug groups according to average %PPI. Testing began a week later. Animals received either APO (0.5 mg/kg, sc) or vehicle (0.01% ascorbic acid) immediately before testing. Tests lasted approximately 19 min and included 5 min of 70 dB(A) background followed by six trial types: PULSE (120 dB(A) 40 ms noise burst), prepulse trials (5 ms noise burst, 15 dB above background followed 10, 20, 30, 60, or 120 ms later by PULSE), and a NOSTIM trial (no stimulus delivery). Seven days later, testing was repeated with APO and vehicle treatment reversed, and treatment order balanced within and between rat strains.

Behavioral Data Analysis

One LE rat displayed negligible startle and was excluded from analyses. PPI was calculated as 100 - ((startle amplitude on prepulse trials/startle amplitude on PULSE trials) × 100), and analyzed by ANOVA with strain as a between-factor and drug and prepulse interval as within-factors. Post-hoc comparisons utilized the Fishe's PLSD test. A measure of the magnitude of the APO effect (mean PPI after vehicle minus mean PPI after APO) was also used for strain comparisons; this value was previously shown effective in detecting differences in PPI drug sensitivity [16,21].

GeneChip Experiments

A 14 d interval was used to insure both drug washout and a diminution of acute stress effects resulting from startle testing. As a result, this study compared basal gene expression in SD vs. LE strains, and not drug-induced gene activation patterns. Fourteen days after completion of PPI testing, animals were anesthetized (15-30 sec) with isoflurane, decapitated, and their brains were removed and placed in ice-cold saline for 30 sec. A 2 mm thick coronal tissue slab was cut with a wire tissue slicer and the NAC was removed bilaterally with a 2.5 mm diam. tissue punch and snap frozen in liquid nitrogen. Total RNA was isolated from tissue using RNeasy columns (Qiagen, Chatsworth, CA). RNA quality was checked by Agilent Lab-on-a-chip and spectrophotometry (260/280). Aliquots of total RNA (5 μg) were used to prepare cDNA. cDNA synthesis, cRNA amplification, hybridization to Affymetrix 230 2.0 Genechips and subsequent washes and scanning (NIH Microarray Consortium) were performed according to the Affymetrix standard protocols (http://www.affymetrix.com/support/technical/manuals.affx).

DNA Microarray Data Analysis

SD vs. LE expression patterns were compared across a focused set of approximately 700 of the > 31,000 transcripts represented on the Affymetrix 230 2.0 rat chip. This focused set was selected prior to the onset of testing, to include genes that are: 1) implicated in the control of either DA-related signal transduction pathways or DA metabolism; 2) regulated by DA agonists, antagonists, or DA depletion, or 3) most strongly associated with schizophrenia, based on published findings.

Raw array images were analyzed, with features extracted using GCOS 1.4 (Affymetrix, Foster City, CA). The resulting CEL files containing probe level information were normalized and converted to gene intensity values by the Robust Analysis of Microarrays (RMA) algorithm [28] with Gene Expression Console (Affymetrix). T-tests (unequal variation) were performed on these normalized values (GeneSpring GX, Agilent Technologies, Santa Clara, CA). Because corrections for multiple comparisons such as Bonferroni are very conservative, p values were chosen using both a very stringent ($p<0.001$) and a more commonly used [26,29], less restrictive level ($p<0.01$) to limit the number of false positives and negatives. Corresponding q-values were calculated, with significance analysis of microarrays (SAM) [30]. The q-value

measures significance by approximating false discovery rates (FDRs) rather than false positive rates [31].

Hierarchical Cluster Analysis (GeneSpring GX) was performed, using genes that exhibited significant expression differences between SD and LE rats. Pearson correlations were used to 1) cluster genes that share common expression patterns into nearby places or branches in the gene tree and, 2) cluster samples with similar expression patterns in the condition tree. Canonical pathways were generated using Ingenuity Pathways Analysis (Ingenuity ® Systems, www.ingenuity.com). Right-tailed Fischer's exact test was used to calculate p-values determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone. Netaffx (Affymetrix, Santa Clara, CA), Ingenuity, and Genespring GX were used to annotate genes.

Because PPI values were not normally distributed across both strains (based on high sensitivity in one and low sensitivity in another strain), Spearman correlations were performed for both baseline PPI and APO effects vs. gene expression values for differentially expressed genes for individual animals in each strain. Prepulse intervals of 10, 20 and 120 ms were used in these correlational analyses, because these intervals are characterized by the most robust strain differences in APO sensitivity (LE most sensitive at 10-20 ms, and SD most sensitive at 120 ms). Pearson correlations were performed on the DNA microarray and real time RT-PCR expression values for several genes.

Taqman RT-PCR

First strand cDNA was synthesized using Quantiscript Reverse transcriptase (Qiagen, Chatsworth, CA). For each sample, one μg of total RNA was reverse transcribed in a 20 μl reaction with a 50 ng mixture of poly A and random hexamer primers, Quantiscript RT buffer, ribonucleotides and Rnase H according to the manufacturer's protocol. cDNAs representing the level of RNA expression were amplified by real time RT-PCR using Applied Biosystems' TaqMan Gene Expression Assays and performed on an Applied Biosystems 7300 in a 20 μl reaction with Universal PCR Master Mix (without AmpErase® UNG) according to the manufacturer's protocol. Genes and assay ID numbers were: – catechol-O-methyl transferase (*Comt*), Rn00561037_m1; for phosphoinositol 3-kinase (*Pi3k*) Rn01406588_m1; for phosphodiesterase 10A (*Pde10A*), Rn00573741_m1; and mitogen associated protein kinase (*Mapk1*) Rn00587719_m1. Gyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) primer/probe sequence was custom designed by the CFAR core and included: 1) TGA CTC TAC CCA CGG CAA GTT, 2) TTC CCG TTG ATG ACC AGC TT, and 3) ACG GCA CAG TCA AGG CTG AGA ATG G. Each assay was performed in triplicate, Ct values were converted to a linear scale, and the mean for each triplicate determined. *Gapdh* was selected for normalization based on having minimal variance in expression across all animals in both strains as detected by the Affymetrix chip. The result for each experimental triplicate was normalized to this corresponding averaged control value to produce RT-PCR values. These values in SD and LE rats were analyzed by independent one-tailed t-tests.

Results

Behavior

PPI data are displayed in Figure 1. ANOVA revealed a significant main effect of strain ($F(1,21)=5.302$, $p<0.05$) and prepulse interval ($F(4,21)=28.723$, $p<0.0001$). In addition, there were significant interactions of APO × strain ($F(1,21)=18.261$, $p<0.001$) and APO × prepulse interval ($F(4,84)=28.55$, $p<0.0001$). There were no significant strain differences in PPI among vehicle-treated rats at any prepulse interval. Post-hoc comparisons revealed significant PPI-*disruptive* effects of APO in SD rats at 30, 60 and 120 ms prepulse intervals (p's<0.01, 0.001

and 0.005, respectively) and in LE rats at 60 ms prepulse intervals ($p<0.05$). Significant PPI-enhancing effects of APO were detected in LE rats at 10 and 20 ms prepulse intervals (p's<0.005 and 0.01, respectively), and in SD rats at 10 ms prepulse intervals ($p<0.01$).

Analysis of startle magnitude on PULSE trials (Figure 1, Inset) revealed a significant main effect of APO ($F(1,21)=20.696$, $p<0.001$). However, there was no significant APO × strain interaction. Post hoc analyses revealed that APO significantly elevated startle magnitude in both SD and LE rats ($p<0.01$, both comparisons). As reported previously [17,19,21], APO effects on startle magnitude did not contribute to the observed strain differences in PPI APO sensitivity: identical strain differences in PPI APO sensitivity were present in a subgroup of SD and LE rats that exhibited no APO-induced increases in startle magnitude.

Gene expression analyses

Several methods were used to confirm that all RNA probes and DNA chips were of the highest quality. The 3′/5′ ratio for the house keeping gene *Gapdh* was >0.99 and <1.00 and for Beta Actin >1.09 and <1.10 for all samples, indicating a high level of transcription across both transcripts with minimal variation across samples. Background values were <67 for all 23 chips, considered to be acceptable background hybridization (Affymetrix). In addition, all spiked controls fell within the optimal range of expression according to parameters set by Affymetrix.

Sixty-one transcripts exhibited significantly different expression at the $p<0.001$ level, and 125 transcripts exhibited significantly different expression at the $p<0.01$ level, in SD vs. LE rats. After significant expression differences in multiple transcripts representing the same gene, and those that exhibited both significantly increased and decreased expression in the same strain were removed, there were 50 genes at the $p<0.001$ (Table 1) and 104 genes at the $p<0.01$ level (Table 2) that exhibited significant expression differences between these rat strains. Many of the differences in Table 1 were very robust, with p values $<10^{-7}$. For all genes included in Table 1, SAM analysis revealed q = 0 % except for Scn7a where q=0.43%. Table 2 lists SAM-derived q-values.

Hierarchical Cluster Analysis of genes that exhibited significant between strain expression differences of $p<0.001$ is displayed in Figure 2. Genes that exhibited more highly correlated levels of expression are clustered together, suggesting co-regulation. This “heat map” clearly demonstrates robust differences in gene expression between SD and LE rats.

Gene expression differences were over-represented in specific canonical pathways. For example, genes differing between SD and LE rats were significantly over-represented among pathways for DA receptor signaling ($p=1.63 \times 10^{-6}$; including *Comt*, dopamine D1 receptor (*Drd1a*), adenylate cyclase 4 (*Adcy4*) and *Adcy5*, protein phosphatase I (*Ppp1*), *Pde10a* and protein kinase A gamma subunit (*Prkag2*) (Figure 3 - Ingenuity® Systems), synaptic long-term potentiation ($p=4.3 \times 10^{-12}$; including AMPA1 receptor (*Gria1*), NMDA receptor subunit 2A (*Grin2b*), glutamate receptor, metabotropic 5 (*Grm5*), phospholipase C (*Plc*) and protein kinase C (*Pkc*); (Figure 4, Ingenuity® Systems) and inositol phosphate metabolism ($p=5.29 \times 10^{-15}$; Table 3, Ingenuity® Systems).

Gene Expression/PPI Correlations

Correlational analyses to test associations between gene expression and PPI included only genes that exhibited significant strain differences, and PPI at prepulse intervals where strain differences were most robust (10, 20 and 120 ms). As seen in Table 4, many of the genes in DA receptor signaling, synaptic long-term potentiation and inositol triphosphate metabolism exhibited significant correlations with PPI APO sensitivity in either or both SD and LE rats.

For example, 6/13 (46%) of the significant genes included from the synaptic long-term potentiation pathway and 5/16 (31%) of the significant genes from the inositol phosphate metabolism pathway exhibited significant correlations with measures of PPI APO sensitivity. *Adcy 4* was the only gene for which expression correlated significantly with PPI sensitivity at both 120 ms intervals in SD rats ($R=0.699$, $p<0.05$) and 20 ms interval in LE rats ($R=0.80$, $p<0.01$) -- the prepulse intervals characterized by the greatest strain differences in PPI APO sensitivity (Figure 1) [18,32].

Validation by Taqman Real-time RT- PCR

Comt, Pik3r1, Pde10a and *Mapk1* were selected from the between strain gene expression differences for validation by Taqman real time RT-PCR. Highly significant differences between SD and LE rats for *Comt* and Pik3r1 were validated by RT-PCR ($p=1.36 \times 10^{-8}$ and $p=3.6 \times 10^{-4}$, respectively) (Figure 5). In addition, microarray and RT-PCR expression values for these genes were significantly correlated (*Comt*: r=0.88, and *Pik3r1*: r=0.81; p's<0.001). However, the significant differences (30% for both genes) detected by the DNA chips for *Pde10a* and *Mapk1* between SD and LE rats were detected as non-significant trends, consistent with the direction of change detected by DNA microarray (data not shown). These data suggest that a 30% difference in expression was too small to be reliably detected as a significant difference by real time RT-PCR, most likely reflecting the greater sensitivity of the DNA chip compared to real time RT-PCR.

Discussion

The present findings confirm reports that SD rats are more sensitive to the PPI-disruptive effects of APO at long prepulse intervals and less sensitive to the PPI-facilitating effects of APO at short prepulse intervals, compared to LE rats [17-19]. To delineate the G-protein coupled, as well as other, signal transduction mechanisms that accompany these strain differences, this study assessed the expression within the NAC of a focused set of approximately 700 transcripts in SD and LE rats associated with DA-regulated signal transduction and mRNA expression, and genes reproducibly associated with schizophrenia. Analyses revealed 104 significant ($p<0.01$) NAC gene expression differences between these strains. Many of these genes are included in canonical signal transduction and metabolic pathways associated with PPI, e.g., DA receptor signaling, synaptic long-term potentiation and inositol phosphate metabolism, as discussed below.

DA receptor signaling

Converging evidence suggests that PPI is regulated by DA-mediated signal transduction pathways in the NAC [21,22,32,33]. Thus, it is not surprising that strain differences in sensitivity to the gating-disruptive effects of APO are accompanied by differential gene expression in DA receptor signaling pathways. For example, differences in *Drd1a*, *Adcy4/5* and *Pde10a* expression would likely impact cAMP levels in NAC medium spiny neurons. Phosphodiesterse inhibitors such as rolipram significantly elevate PPI levels, suggesting that PPI is modulated by variations in cellular cAMP levels [34]. In this regard, it is of considerable interest that: 1) both *Adcy 4* and *Adcy5* expression levels exhibited significant strain differences; and 2) *Adcy 4* was the only gene in which mRNA expression significantly correlated with measures of PPI APO sensitivity in both SD and LE rats, at prepulse intervals where strain differences in PPI APO sensitivity were most robust. Specifically, significant positive correlations were detected between *Adcy 4* expression and PPI APO sensitivity at 120 ms intervals in SD rats (where their PPI-*reducing* effects of APO are most distinct from LE rats) and between *Adcy 4* expression and PPI APO sensitivity at 20 ms intervals in LE rats (where their PPI-*enhancing* effects of APO are most distinct from SD rats). These data suggest

that strain differences in *Adcy 4* expression are associated with the observed strain differences in sensitivity to the effects of APO on PPI, at both short and long prepulse intervals.

Comt exhibited the most significant strain differences in expression, among all genes analyzed (greater than 3 fold higher expression in the LE rats). *Comt* plays a major role in DA metabolism and has been associated with schizophrenia [35]. In humans, the COMT gene contains a functional polymorphism, that results in two common variants of the enzyme - *val* and *met* - the former being associated with higher enzyme activity. This polymorphism appears to impact both DA-regulated prefrontal cortical activity and neurocognitive performance, with the *val* polymorphism (and presumably higher enzyme activity) most often associated with schizophrenia, as well as poorer neurocognitive performance (cf.[36]). Perhaps more directly relevant to the present findings, increased COMT activity may also be associated with *increased* dopaminergic activity in midbrain DA neurons that innervate the striatum and NAC [37]. Such a mechanism is consistent with direct measurements of NAC DA and metabolites [20], which demonstrated greater NAC and striatal DA turnover in LE vs. SD rats, and intermediate levels of NAC DA turnover among F1 (SD × LE) rats. Based on these differences in basal (but not drug-stimulated) DA turnover, we had previously speculated that brain mechanisms involved in the DAergic regulation of PPI may have "compensated" for elevated DA turnover in LE rats, resulting in a decreased sensitivity to the gating-disruptive impact of DA agonists. Conversely, the "set point" for the DAergic regulation of PPI in SD rats, with relatively lower levels of basal DA turnover, might render them more sensitive to the gating-disruptive effects of DA agonists. While the present findings with *Comt* may help clarify the basis for strain (and apparently heritable) differences in NAC DA turnover in SD and LE rats, the relationship between these differences and differential PPI APO sensitivity remains a matter for speculation.

Synaptic Long-Term Potentiation

Many significant strain differences were detected in the expression of genes that contribute to the synaptic long-term potentiation pathway. Synaptic long-term potentiation is a prominent characteristic of AMPA, NMDA and glutamate metabotropic receptor signaling pathways. Converging evidence suggests that DA/glutamate interactions in the NAC contribute to the regulation of PPI [38,39]. Past findings suggest that AMPA receptors in the NAC core regulate PPI via a pro-dopaminergic effect at presynaptic DA terminals, while AMPA receptor activation in the NAC shell disrupts PPI independent of dopaminergic transmission [38]. Others have reported that PPI is disrupted after intra-NAC core infusion of either NMDA or non-competitive NMDA receptor antagonists [39,40]. In the present study, both *Grin2a* and *Gria1* expression in SD rats, but not LE rats, significantly correlated with measures of PPI APO sensitivity, and *Grm5* expression significantly correlated with measures of PPI APO sensitivity in LE, but not SD rats. Furthermore, 46% of the differentially expressed genes examined in this pathway exhibited significant correlations with the prepulse intervals of interest, suggesting a role for this pathway in the differential sensitivity to APO-mediated PPI deficits in SD vs. LE rats.

Previous reports are consistent with a role for *Grm5* in the development of PPI deficits. Brody et al. reported that *Grm5* knockout mice exhibit robust PPI deficits [41]. Mice lacking *Homer1*, a gene that regulates Grm5 post synaptic activity [42], also exhibit PPI deficits [43]. Grm5 interacts with Gq to transduce glutamatergic signals through phospholipase C, resulting in increased inositol triphosphate and calcium release from the endoplasmic reticulum [44]. Therefore, Grm5 contributes to both synaptic long-term potentiation and inositol phosphate metabolism, and might impact strain differences in PPI APO sensitivity via either or both pathways.

Inositol Phosphate Metabolism

Genes encoding proteins that regulate inositol phosphate metabolism were highly represented among the NAC genes differentially expressed in SD vs. LE rats. Inositol phosphate metabolism is regulated via Grm5 and D1 receptors, among other mechanisms [45,46]. Grottick et al. [47] reported significant expression differences in *Grm5*, inositol monophosphatase (*Impa*) and *Plc* in the prefrontal cortex of mouse substrains that differ in baseline PPI. Conceivably, strain differences in NAC gene expression within this metabolic pathway may contribute to the differential sensitivity to the PPI-disruptive effects of APO.

Inositol monophosphatase 2 (*Impa2*), one of the genes in this pathway that is differentially regulated in SD and LE rats, is associated with bipolar disorder and with the therapeutic effects of lithium [48-50]. The precise PPI phenotype in bipolar disorder remains an issue of some dispute. While PPI has been reported to be deficient in acutely psychotic patients with bipolar disorder [51] and in unaffected siblings of bipolar patients [52], it has also been reported to be normal in manic, mixed episode and euthymic adults and children with bipolar disorder [53-55].

Interpretation of strain differences in mRNA expression must be done with caution. Although changes in mRNA levels have been shown to translate into changes in protein levels [56,57], there are reports of a dissociation of transcription and translation [58]. In addition, it may be premature to exclude from analysis the genes that do not exhibit between strain differences in mRNA expression, as they may still contribute to the differential APO sensitivity phenotype via strain differences in cellular processes such as post translation modifications, changes in binding affinity, enzymatic activity or intracellular trafficking. For example, NAC D2 receptor mRNA was not differentially expressed in SD vs. LE rats, despite the fact that these strains differ in NAC DA-stimulated (but not basal) [^{35}S]GTPγS binding [21], thought to reflect the efficacy of D2-like receptor-G-protein coupling.

The present findings suggest that a phenotype of elevated sensitivity to the gating-disruptive effects of DA neurotransmission might reflect the action of many different genes - perhaps dozens or more - working alone or in concert. It is thus conceivable that reduced PPI in disease states might also reflect the presence of many genes of differing effects. Perhaps most striking in the present data is the common residence of a disproportionate number of these genes within a small number of important pathways; these pathways may exert substantial influence on phenotypes of impaired gating, and may thus represent important targets for therapeutic interventions. This general strategy of exploring these and related pathways for potential receptor-independent antipsychotics and pro-cognitive agents is already being applied to phosphodiesterase inhibitors [34], COMT [59] and other enzymes, and signal-regulating molecules.

In summary, rat strains that exhibit heritable differences in PPI APO sensitivity also differ significantly in NAC gene expression within DA receptor signaling, synaptic long-term potentiation and inositol phosphate metabolic pathways. With of some genes, such as *Comt*, there was no overlap in NAC expression levels among rats from these two strains. In other cases, expression levels correlate significantly with PPI APO sensitivity in one or both strains. An understanding of the molecular mechanisms underlying strain differences in DA-mediated gating deficits should facilitate the identification of pathological mechanisms and novel therapeutic avenues in specific neuropsychiatric disorders -- such as schizophrenia and Tourette Syndrome -- that are also characterized by heritable PPI deficits.

Acknowledgments

NRS, JMS, PDS and RLS were supported by NIMH/NIH R01 MH68366-04. There are no conflicts of interest. NRS serves as a paid scientific consultant to Allergan Pharmaceuticals, and received research support from Pfizer Pharmaceuticals. There are no other biomedical financial disclosures. We thank the UCLA Microarray Core division of the NIH Microarray Consortium for their expression profiling services. We also thank the University of California San Diego, Center for AIDS Research Genomics Core Laboratory (Director, Dr. Christopher Woelk; Grant number, 5P30 AI36214) and the San Diego Veterans Medical Research Foundation for performing the real time RT-PCR assays. We are grateful to Michelle Brier, Adrienne B. Mora, David Ko and Alaina C. Neary for their expert technical support.

References

1. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 2003;160:636–45. [PubMed: 12668349]
2. Gould TD, Gottesman II. Psychiatric endophenotypes and the development of valid animal models. Genes Brain Behav 2006;5:113–9. [PubMed: 16507002]
3. Braff DL, Freedman R, Schork NJ, Gottesman II. Deconstructing schizophrenia: an overview of the use of endophenotypes in order to understand a complex disorder. Schizophr Bull 2007;33:21–32. [PubMed: 17088422]
4. Graham FK. Presidential Address, 1974. The more or less startling effects of weak prestimulation. Psychophysiology 1975;12:238–248. [PubMed: 1153628]
5. Anokhin AP, Heath AC, Myers E, Ralano A, Wood S. Genetic influences on prepulse inhibition of startle reflex in humans. Neurosci Lett 2003;353:45–8. [PubMed: 14642434]
6. Greenwood TA, Braff DL, Cadenhead K, et al. The Consortium on the Genetics of Schizophrenia (COGS): Preliminary heritability analyses of endophenotypic measures for schizophrenia. Archives of General Psychiatry. In Press
7. Swerdlow NR, Caine SB, Braff DL, Geyer MA. Neural substrates of sensorimotor gating of the startle reflex: Preclinical findings and their implications. J Psychopharmacol 1992;6:176–190.
8. Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl) 2001;156:194–215. [PubMed: 11549223]
9. Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L. Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 1978;15:339–343. [PubMed: 693742]
10. 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]
11. Swerdlow NR, Light GA, Cadenhead KS, Sprock J, Hsieh MH, Braff DL. Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. Arch Gen Psychiatry 2006;63:1325–35. [PubMed: 17146007]
12. 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]
13. Mansbach RS, Geyer MA, Braff DL. Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berl) 1988;94:507–514. [PubMed: 3131796]
14. 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 2003;75:191–7. [PubMed: 12759127]
15. Swerdlow NR, Platten A, Kim YK, et al. Sensitivity to the dopaminergic regulation of prepulse inhibition in rats: evidence for genetic, but not environmental determinants. Pharmacol Biochem Behav 2001;70:219–26. [PubMed: 11701191]
16. Swerdlow NR, Shoemaker JM, Pitcher L, et al. Genetic differences in startle gating-disruptive effects of apomorphine: evidence for central mediation. Behav Neurosci 2002;116:682–90. [PubMed: 12148935]
17. 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 (Berl) 2004;174:452–62. [PubMed: 15300359]

18. 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 (Berl) 2004;174:441–51. [PubMed: 15300358]
19. 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 2004;77:291–302. [PubMed: 14751457]
20. Swerdlow NR, Kuczenski R, Goins JC, et al. Neurochemical analysis of rat strain differences in the startle gating-disruptive effects of dopamine agonists. Pharmacol Biochem Behav 2005;80:203–11. [PubMed: 15680173]
21. 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;31:721–9. [PubMed: 16123742]
22. 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;1119:203–14. [PubMed: 16979142]
23. Swerdlow NR, Shoemaker JM, Bongiovanni MJ, Neary AC, Tochen LS, Saint Marie RL. Strain differences in the disruption of prepulse inhibition of startle after systemic and intra-accumbens amphetamine administration. Pharmacol Biochem Behav 2007;87:1–10. [PubMed: 17475315]
24. Saint Marie RL, Neary AC, Shoemaker JM, Swerdlow NR. Apomorphine effects on CREB phosphorylation in the nucleus accumbens of rat strains that differ in PPI sensitivity. Biological Psychiatry 2007;61(8S):113.Supplement
25. Castellanos FX, Fine EJ, Kaysen D, Marsh WL, Rapoport JL, Hallett M. Sensorimotor gating in boys with Tourett's syndrome and ADHD: preliminary results. Biol Psychiatry 1996;39:33–41. [PubMed: 8719124]
26. Shilling PD, Kuczenski R, Segal DS, Barrett TB, Kelsoe JR. Differential regulation of immediate-early gene expression in the prefrontal cortex of rats with a high vs low behavioral response to methamphetamine. Neuropsychopharmacology 2006;31:2359–67. [PubMed: 16855532]
27. Palmer AA, Verbitsky M, Suresh R, et al. Gene expression differences in mice divergently selected for methamphetamine sensitivity. Mamm Genome 2005;16:291–305. [PubMed: 16104378]
28. Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4:249–64. [PubMed: 12925520]
29. Sabatini MJ, Ebert P, Lewis DA, L P, Cameron JL, Mirnics K. Amygdala gene expression correlates of social behavior in monkeys experiencing maternal separation. J Neuroscience 2007;27:3295–3304.
30. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA 2001;98:5116–21. [PubMed: 11309499]
31. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci USA 2003;100:9440–5. [PubMed: 12883005]
32. Culm KE, Lugo-Escobar N, Hope BT, Hammer RP Jr. Repeated quinpirole treatment increases cAMP-dependent protein kinase activity and CREB phosphorylation in nucleus accumbens and reverses quinpirole-induced sensorimotor gating deficits in rats. Neuropsychopharmacology 2004;29 (10):1823–30. [PubMed: 15138441]
33. Kelly MP, Isiegas C, Cheung YF, et al. Constitutive activation of Galphas within forebrain neurons causes deficits in sensorimotor gating because of PKA-dependent decreases in cAMP. Neuropsychopharmacology 2007;32:577–88. [PubMed: 16738544]
34. Kanes SJ, Tokarczyk J, Siegel SJ, Bilker W, Abel T, Kelly MP. Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. Neuroscience 2007;144:239–46. [PubMed: 17081698]
35. Egan MF, Goldberg TE, Kolachana BS, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 2001;98:6917–22. [PubMed: 11381111]

36. Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 2005;10:40–68. [PubMed: 15263907]
37. Akil M, Kolachana BS, Rothmond DA, Hyde TM, Weinberger DR, Kleinman JE. Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. J Neurosci 2003;23:2008–13. [PubMed: 12657658]
38. Wan FJ, Swerdlow NR. Sensorimotor gating in rats is regulated by different dopamine-glutamate interactions in the nucleus accumbens core and shell subregions. Brain Res 1996;722:168–176. [PubMed: 8813362]
39. Kretschmer BD, Koch M. The ventral pallidum mediates disruption of prepulse inhibition of the acoustic startle response induced by dopamine agonists, but not by NMDA antagonists. Brain Res 1998;798:204–10. [PubMed: 9666129]
40. Reijmers LG, Vanderheyden PM, Peeters BW. Changes in prepulse inhibition after local administration of NMDA receptor ligands in the core region of the rat nucleus accumbens. Eur J Pharmacol 1995;272:131–8. [PubMed: 7713156]
41. Brody SA, Conquet F, Geyer MA. Disruption of prepulse inhibition in mice lacking mGluR1. Eur J Neurosci 2003;18:3361–6. [PubMed: 14686909]
42. Ango F, Pin JP, Tu JC, et al. Dendritic and axonal targeting of type 5 metabotropic glutamate receptor is regulated by homer1 proteins and neuronal excitation. J Neurosci 2000;20:8710–6. [PubMed: 11102477]
43. Szumlinski KK, Lominac KD, Kleschen MJ, et al. Behavioral and neurochemical phenotyping of Homer1 mutant mice: possible relevance to schizophrenia. Genes Brain Behav 2005;4:273–88. [PubMed: 16011574]
44. Cooper, JR.; Bloom, FE.; Roth, RH. The Biochemical Basis of Neuropharmacology. Vol. 8. New York: Oxford University Press; 2003.
45. Pacheco MA, Jope RS. Comparison of [3H]phosphatidylinositol and [3H]phosphatidylinositol 4,5-bisphosphate hydrolysis in postmortem human brain membranes and characterization of stimulation by dopamine D1 receptors. J Neurochem 1997;69:639–44. [PubMed: 9231722]
46. Mannoury la Cour C, Vidal S, Pasteau V, Cussac D, Millan MJ. Dopamine D1 receptor coupling to Gs/olf and Gq in rat striatum and cortex: a scintillation proximity assay (SPA)/antibody-capture characterization of benzazepine agonists. Neuropharmacology 2007;52:1003–14. [PubMed: 17178132]
47. Grottick AJ, Bagnol D, Phillips S, et al. Neurotransmission- and cellular stress-related gene expression associated with prepulse inhibition in mice. Brain Res Mol Brain Res 2005;139:153–62. [PubMed: 15961183]
48. Cryns K, Shamir A, Shapiro J, et al. Lack of lithium-like behavioral and molecular effects in IMPA2 knockout mice. Neuropsychopharmacology 2007;32:881–91. [PubMed: 16841073]
49. Seelan RS, Parthasarathy LK, Parthasarathy RN. Lithium modulation of the human inositol monophosphatase 2 (IMPA2) promoter. Biochem Biophys Res Commun 2004;324:1370–8. [PubMed: 15504365]
50. Sjoholt G, Ebstein RP, Lie RT, et al. Examination of IMPA1 and IMPA2 genes in manic-depressive patients: association between IMPA2 promoter polymorphisms and bipolar disorder. Mol Psychiatry 2004;9:621–9. [PubMed: 14699425]
51. Perry W, Minassian A, Feifel D, Braff DL. Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. Biol Psychiatry 2001;50:418–24. [PubMed: 11566158]
52. Giakoumaki SG, Roussos P, Rogdaki M, Karli C, Bitsios P, Frangou S. Evidence of Disrupted Prepulse Inhibition in Unaffected Siblings of Bipolar Disorder Patients. Biol Psychiatry. 2007
53. Carroll CA, Vohs JL, O'Donnell BF, Shekhar A, Hetrick WP. Sensorimotor gating in manic and mixed episode bipolar disorder. Bipolar Disord 2007;9:221–9. [PubMed: 17430296]
54. Barrett SL, Kelly C, Watson DR, Bell R, King DJ. Normal levels of prepulse inhibition in the euthymic phase of bipolar disorder. Psychol Med 2005;35:1737–46. [PubMed: 16300689]
55. Rich BA, Vinton D, Grillon C, Bhangoo RK, Leibenluft E. An investigation of prepulse inhibition in pediatric bipolar disorder. Bipolar Disord 2005;7:198–203. [PubMed: 15762862]
56. Cerruti C, Pilotte NS, Uhl G, Kuhar MJ. Reduction in dopamine transporter mRNA after cessation of repeated cocaine administration. Brain Res Mol Brain Res 1994;22:132–138. [PubMed: 8015373]

57. MacDonald ML, Eaton ME, Dudman JT, Konradi C. Antipsychotic drugs elevate mRNA levels of presynaptic proteins in the frontal cortex of the rat. Biol Psychiatry 2005;57:1041–51. [PubMed: 15860345]

58. Mirnics K, Levitt P, Lewis DA. Critical appraisal of DNA microarrays in psychiatric genomics. Biol Psychiatry 2006;60:163–76. [PubMed: 16616896]

59. Apud JA, Mattay V, Chen J, et al. Tolcapone improves cognition and cortical information processing in normal human subjects. Neuropsychopharmacology 2007;32:1011–20. [PubMed: 17063156]

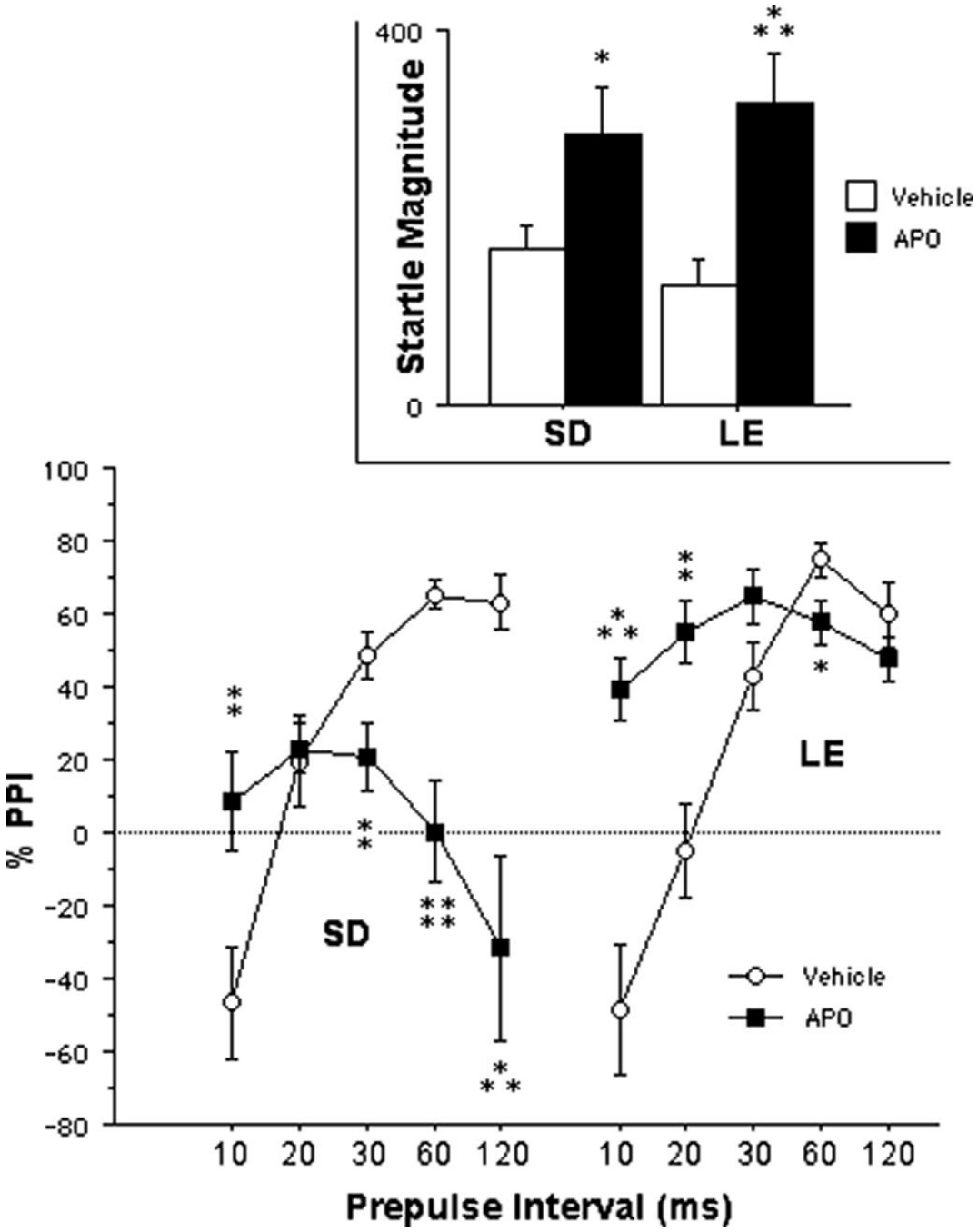

Figure 1.

The effects of APO on PPI at prepulse intervals of 10 - 120 ms in LE and SD rats. Inset shows APO effects on startle magnitude. Significantly different from VEH (same strain) = * $p < 0.05$, ** $p < 0.01$; ⁂ $p < 0.005$; ⁜ $p < 0.001$.

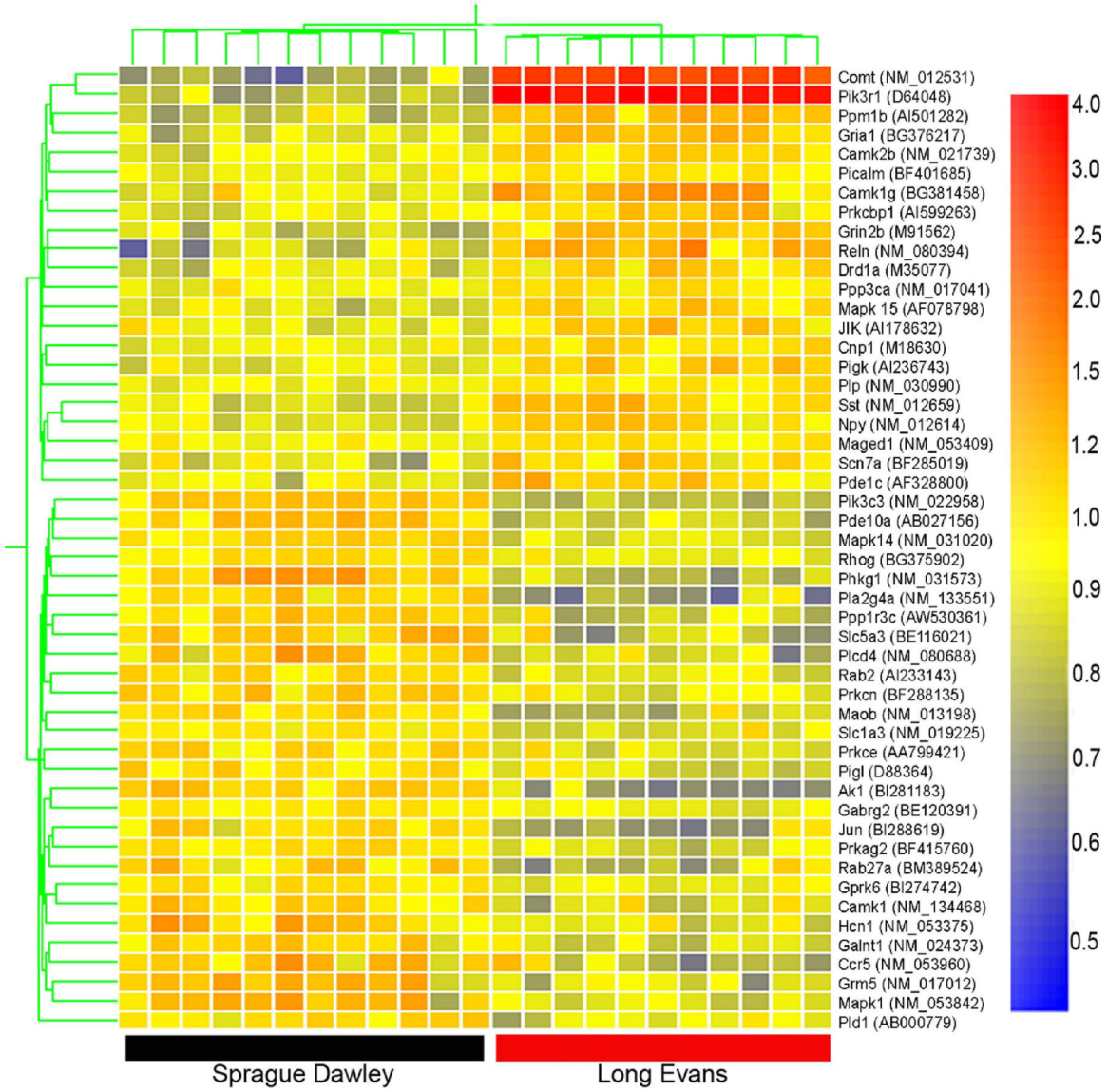

Figure 2.

Hierarchical Clustering Analysis of significant gene expression differences ($p < 0.001$) in the NAC of SD and LE rats, that differed in their sensitivity to the PPI-disruptive effects of the dopamine agonist, apomorphine (0.5 mg/kg). Samples with similar expression patterns are grouped together in a dendrogram. All values normalized by RMA (see Methods) were renormalized to a median value of 1 across all rats. Rows correspond to specific genes and columns correspond to individual rats (SD: left side, LE: right side). Red indicates higher than and blue less than the median gene expression value for each gene (See key).

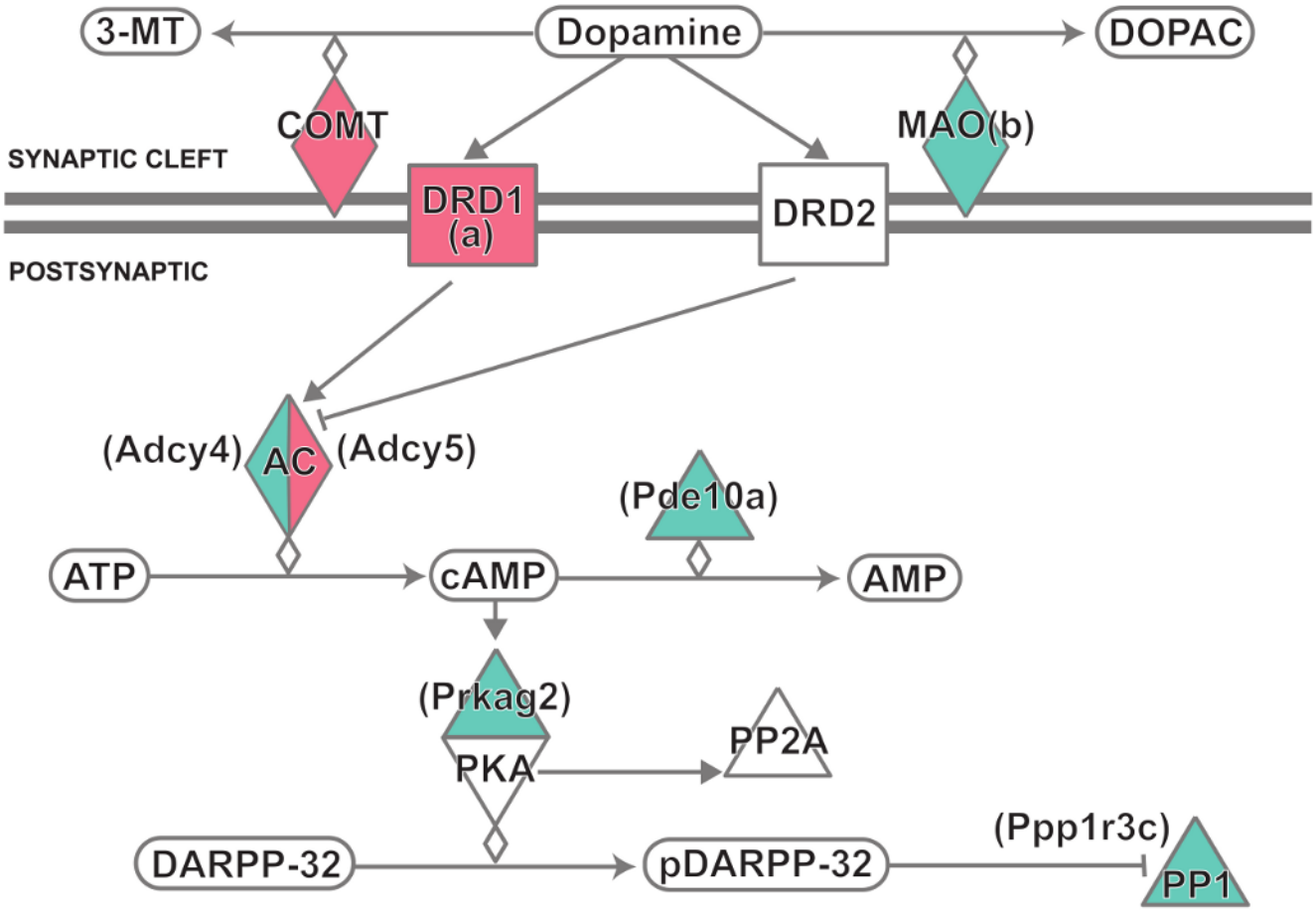

Figure 3.

Dopamine Receptor Signaling: Genes differentially expressed in the NAC of LE vs. SD rats are significantly over represented in the dopamine-receptor signaling pathway. In LE rats, *COMT*, *DRD1a* and *ADCY5* mRNA levels in the NAC (red-filled shapes) are higher than in SD rats. In contrast, *MAO(b)*, *ADCY4*, *PDE10A*, *PKA (Prkag2)* and PP1 (*Ppp1r3c*) (green-filled shapes) exhibit reduced expression in LE rats compared to SD rats. Adapted from Ingenuity ® System's database of canonical pathways for Dopamine Receptor Signaling. Where the biochemical annotation in the Ingenuity canonical pathway differs from the gene symbol used by Affymetrix (see Table 1), the Affymetrix gene symbol is included in parentheses.

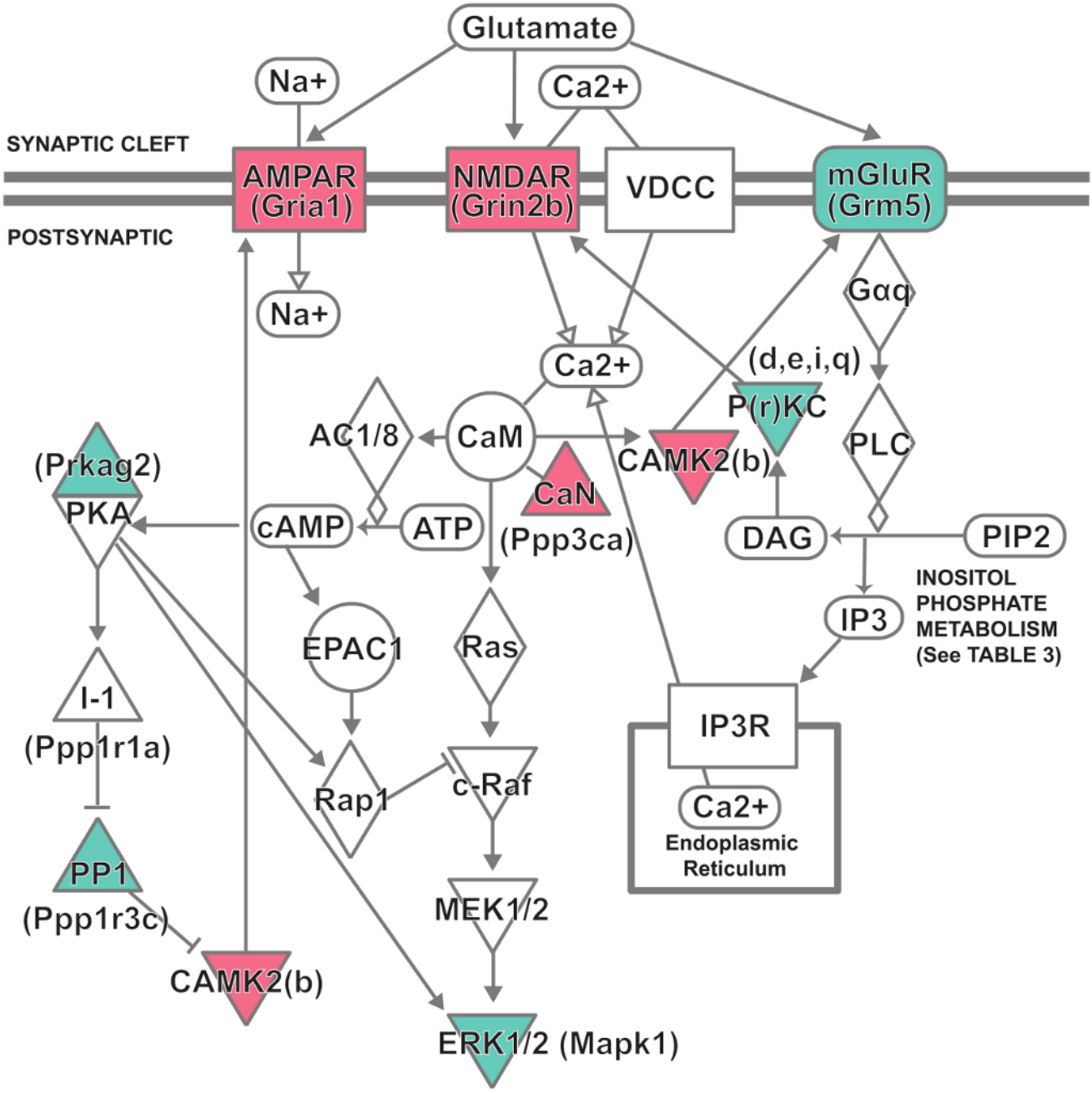

Figure 4.

Synaptic Long-Term Potentiation: Genes differentially expressed in the NAC of LE vs. SD rats are significantly over represented in the synaptic long-term potentiation pathway. In LE rats, genes that represent the ionotropic AMPA (*Gria1*) and NMDA (*Grin2b*) classes of glutamate receptors exhibit significantly higher mRNA expression levels (red-filled shapes) compared to SD rats, as do *CAMK2* and CaN (*Ppp3ca*). Conversely, genes coding for the metabotropic glutamate receptor mGluR (*Grm5*) are expressed at higher levels in SD compared to LE rats (green-filled shapes), as are several genes for enzymes in this pathway, e.g., *PKA (Prkag2), PP1 (Ppp1r3c), CAMK2(b), P(r)KC* (*delta, epsilon, iota and theta subunits*), and *MAPK1*. Adapted from Ingenuity® System's database of canonical pathways for Synaptic

Long-Term Potentiation. Where the biochemical annotation in the Ingenuity canonical pathway differs from the gene symbol used by Affymetrix (see Tables 1 & 2), the Affymetrix gene symbol is included in parentheses.

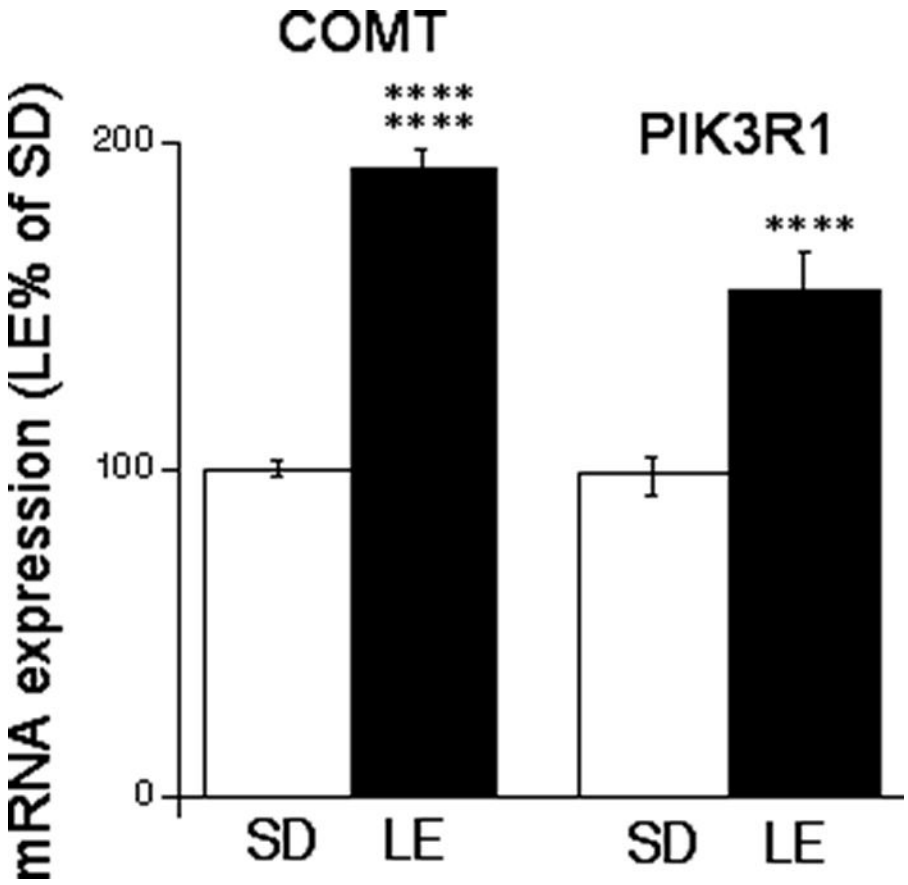

Figure 5.
Real time RT-PCR for *COMT* and *PIK3R1*. Expression values were normalized to *GAPDH*. Values represent the mean percentage difference from SD rats ± SEM. **** ($p < 0.001$), and ($p < 10^{-7}$).

Table 1

Significant Gene Expression Differences, LE vs. SD, P < 0.001

For all genes in this table the q-values as determined by SAM = 0 %, except for SACN7A where q = 0.43 %

Accession #	Genes	Symbol	P–Value	LE/SD
	P < 0.0000001			
NM_012531	Catechol-O-methyl transferase	Comt	4.81×10^{-17}	3.27
D64048	Phosphatidylinositol 3-kinase, regulatory subunit	Pik3r1	1.94×10^{-14}	4.51
NM_022958	Phosphoinositide-3-kinase, class 3	Pik3c3	1.43×10^{-11}	0.74
AI501282	Protein phosphatase 1B, magnesium dependent, beta isoform	Ppm1b	2.18×10^{-8}	1.38
NM_031020	Mitogen activated protein kinase 14	Mapk14	6.19×10^{-8}	0.86
	P < 0.000001			
BI281183	Adenylate kinase 1*	Ak1	1.43×10^{-7}	0.68
M91562	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	Grin2b	2.09×10^{-7}	1.30
AB027156	Phosphodiesterase 10A*	Pde10a	3.96×10^{-7}	0.77
	P < 0.00001			
BG376217	Glutamate receptor, ionotropic, AMPA1 (alpha 1)	Gria1	1.13×10^{-6}	1.27
BG375902	Ras homolog gene family, member G	Rhog	4.22×10^{-6}	0.91
NM_031573	Phosphorylase kinase gamma 1	Phkg1	4.43×10^{-6}	0.68
NM_080394	Reelin*	Reln	7.84×10^{-6}	1.48
	P < 0.0001			
NM _133551	Phospholipase A2, group IVA (cytosolic, calcium-dependent)	Pla2g4a	0.000011	0.73
NM_012659	Somatostatin	Sst	0.000015	1.24
NM_021739	Calcium/calmodulin-dependent protein kinase II, beta	Camk2b	0.000018	1.13
NM_013198	Monoamine oxidase B	Maob	0.000019	0.81
BI288619	Jun oncogene*	Jun	0.000021	0.76
BF415760	Protein kinase, AMP-activated, gamma 2 non-catalytic	Prkag2	0.000023	0.88
AW530361	Protein phosphatase 1, regulatory (inhibitor) subunit 3C*	Ppp1r3c	0.000028	0.81
BF401685	Phosphatidylinositol binding clathrin assembly protein	Picalm	0.000029	1.07
AI233143	RAB2, member RAS oncogene family-like	Rab2	0.000035	0.81
AA799421	Protein kinase C, epsilon	Prkce	0.000036	0.87
M18630	Cyclic nucleotide phosphodiesterase 1*	Cnp1	0.000040	1.11
BE116021	Solute carrier family 5 (inositol transporters), member 3	Slc5a3	0.000057	0.76
AF078798	Mitogen activated protein kinase 15	Mapk15	0.000068	1.12
NM_030990	Proteolipid protein	Plp	0.000077	1.07
BI274742	G protein-coupled receptor kinase 6	Gprk6	0.000091	0.92
NM_024373	UDP-N-acetyl-alpha-D-galactosamine:polypeptide acetylgalactosaminyltransferase 1	Galnt1	0.000092	0.85
D88364	Phosphatidylinositol glycan, class L	Pigl	0.000093	0.87
AI236743	Phosphatidylinositol glycan, class K	Pigk	0.000094	1.19
	P < 0.001			
BF288135	Protein kinase C, nu	Prkcn	0.00010	0.86
NM_012614	Neuropeptide Y	Npy	0.00010	1.18
BE120391	Gamma-aminobutyric acid A receptor, gamma 2	Gabrg2	0.00011	0.94
BG381458	Calcium/calmodulin-dependent protein kinase I gamma*	Camk1g	0.00011	1.41
NM_017012	Glutamate receptor, metabotropic 5	Grm5	0.00020	0.78
NM_080688	Phospholipase C, delta 4	Plcd4	0.00021	0.78
NM_053842	Mitogen activated protein kinase 1	Mapk1	0.00024	0.77
M35077	Dopamine receptor D1A	Drd1a	0.00029	1.16
BM389524	RAB27A member RAS oncogene family*	Rab27a	0.00031	0.79
AI178632	JNK/SAPK-inhibitory kinase	JIK	0.00038	1.14

Accession #	Genes	Symbol	P–Value	LE/SD
NM_053960	Chemokine (C-C motif) receptor 5	Ccr5	0.00041	0.77
NM_017041	Protein phosphatase 3, catalytic subunit, alpha isoform	Ppp3ca	0.00042	1.07
AF328800	Phosphodiesterase 1C	Pde1c	0.00048	1.19
BF285019	Sodium channel, voltage-gated, type VII, alpha	Scn7a	0.00059	1.18
NM_134468	Calcium/calmodulin-dependent protein kinase I	Camk1	0.00066	0.85
NM_053409	Melanoma antigen, family D, 1	Maged1	0.00084	1.05
NM_019225	Glial high affinity glutamate transporter, member 3	Slc1a3	0.00083	0.91
AB000779	Phospholipase D1	Pld1	0.00091	0.89
AI599263	Protein kinase C binding protein 1	Prkcpb1	0.00095	1.16
NM_053375	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1	Hcn1	0.00095	0.80

* indicates genes detected as significantly different multiple times

Table 2

Significant gene expression differences, LE vs. SD, P < 0.01 and > 0.001

*indicates genes detected as significantly different multiple times

Accession #	Genes	Symbol	P-Value	q-Value (%)	LE/SD
	P < 0.01 and > 0.001				
NM_133289	Sodium channel, voltage-gated, type IX, alpha	Scn9a	0.0011	0	1.40
AF402786	Fms-related tyrosine kinase 4	Flt4	0.0012	0.91	1.11
AF400662	Calcium channel, voltage-dependent, alpha2/delta subunit 1	Cacna2d1	0.0012	0	0.88
NM_012877	Sodium channel, voltage-gated, type II, beta	Scn2b	0.0014	0	1.22
NM_020084	Calcium channel, voltage-dependent, alpha 1 I subunit	Cacna1i	0.0017	0.43	1.12
BG380493	Multiple inositol polyphosphate histidine phosphatase 1	Minpp1	0.0018	0	1.10
AW525609	Inositol (myo)-1(or 4)-monophosphatase 2	Impa2	0.0018	0.43	1.18
BF282264	cAMP responsive element binding protein 3-like 3	Creb3l3	0.0019	0.43	1.22
AA799542	Ras-related C3 botulinum toxin substrate 1	Rac1	0.0019	0	0.97
NM_053978	RAB28, member RAS oncogene family	Rab28	0.0020	0	0.96
BF284699	Protein kinase C, iota	Prkci	0.0023	0	0.94
AW532988	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2	Hcn2	0.0025	0.43	1.07
BE127432	Ras homolog gene family, member J	Rhoj	0.0028	0.55	1.11
NM_017289	Gamma-aminobutyric acid A receptor, delta*	Gabrd	0.0028	0.43	1.09
NM_022542	Ras homolog gene family, member B	Rhob	0.0028	0	1.09
NM_022944	Inositol polyphosphate phosphatase-like 1	Inppl1	0.0030	0.43	0.90
NM_021660	Inositol hexaphosphate kinase 2	Ihpk2	0.0032	0.43	0.92
AI454542	MAM domain containing glycosylphosphatidylinositol anchor 2	Mamdc1	0.0033	0.43	0.91
NM_033376	Potassium channel, subfamily K, member 3	Kcnk3	0.0034	0.55	1.09
NM_133307	Protein kinase C, delta	Prkcd	0.0036	0.55	0.84
BI283843	Mitogen activated protein kinase kinase 3	Map2k3	0.0036	0.43	0.93
NM_031338	Calcium/calmodulin-dependent protein kinase kinase 2, beta	Camkk2	0.0037	0	0.82
AI411439	RAB18, member RAS oncogene family	Rab18	0.0040	0.43	0.92
NM_021589	Neurotrophic tyrosine kinase, receptor, type 1	Ntrk1	0.0041	0.55	1.15
AF194443	Neuregulin 1	Nrg1	0.0043	0.55	1.19
BI275516	Inositol polyphosphate-1-phosphatase	Inpp1	0.0043	0.43	0.92
BF288347	Potassium channel modulatory factor 1	Kcmf1	0.0047	0.91	1.08
L14447	Neurotrophic tyrosine kinase, receptor, type 3	Ntrk3	0.0049	0.55	1.11
AI556291	Potassium channel, subfamily T, member 1	Kcnt1	0.0049	1.60	1.10
AA851587	Serum response factor binding protein 1	Srfbp1	0.0055	0.55	1.08
NM_022600	Adenylate cyclase 5	Adcy5	0.0059	0.55	1.16
NM_019363	Aldehyde oxidase 1	Aox1	0.0060	0.55	0.85
AI575972	Mitogen-activated protein kinase 8 interacting protein 2	Mapk8ip2	0.0060	0.91	1.15
NM_012551	Early growth response 1	Erg1	0.0062	0.55	0.88
AI171093	Protein kinase C, theta	Prkcq	0.0062	0.55	1.09
X63744	Glial high affinity glutamate transporter	Slc1a3	0.0065	0.55	1.10
BI289129	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1	Tie1	0.0066	0.91	1.16
D88672	Phospholipase D2	Pld2	0.0068	0.91	0.90
BF406240	FMS-like tyrosine kinase 3	Flt3	0.0071	0.91	1.10
AI102103	Phosphatidylinositol 4-kinase, catalytic, beta polypeptide	Pik4cb	0.0072	0.55	1.05
AA849961	Ras homolog gene family, member Q	Rhoq	0.0076	0.55	0.89
NM_053806	Potassium channel, subfamily K, member 6	Kcnk6	0.0076	4.36	0.91
U54699	Sodium channel, nonvoltage-gated 1 alpha	Scnn1a	0.0077	4.36	1.08
NM_030871	Phosphodiesterase 1A, calmodulin-dependent	Pde1a	0.0078	0.91	1.19
AF361340	Calcium channel, voltage-dependent, gamma subunit 3	Cacng3	0.0078	0.55	1.10
NM_133590	RAB7, member RAS oncogene family-like 1	Rab7l1	0.0078	0.91	0.88
BI285710	Potassium channel tetramerisation domain containing 1	Kctd1	0.0081	0.43	0.90

Accession #	Genes	Symbol	P-Value	q-Value (%)	LE/SD
BE100607	Phosphatidylinositol transfer protein, membrane- associated 1	Pitpnm1	0.0083	0.55	1.04
BE102996	Neurotrophic tyrosine kinase, receptor, type 2	Ntrk2	0.0084	0.91	0.90
BG380938	Diacylglycerol kinase, iota	Dgki	0.0085	0.91	1.11
BI275870	RAB11b, member RAS oncogene family	Rab11b	0.0089	0.91	1.03
BE118560	RAB11a, member RAS oncogene family	Rab11a	0.0092	0.91	0.93
AF343575	Parkin	Park2	0.0095	1.60	0.85
NM_019285	Adenylate cyclase 4	Adcy4	0.0096	2.82	0.93
BF281311	Casein kinase 2, beta subunit	Csnk2b	0.0097	0.91	0.95

Table 3

Differentially expressed genes (LE vs. SD) that are included in Inositol Phosphate Metabolism were significantly over represented ($p = 5.29 \times 10^{-15}$)

Adapted from Ingenuity ® System's database of canonical pathways

Table 3A SD gene expression > LE gene expression		
Accession #	**Gene**	**Symbol**
NM_053960	Chemokine receptor 5	Ccr5 **
BI274742	G protein-coupled receptor kinase 6	Gprk6 ***
NM_021660	Inositol hexaphosphate kinase 2	Ihpk2 *
BI275516	Inositol polyphosphate-1-phophatase	Inpp1 *
NM_022944	Inositol polyphosphate phophatase-like 1	Inppl1 *
BI283843	Mitogen-activated protein kinase kinase 3	Map2k3 *
NM_053842	Mitogen-activated protein kinase 1	Mapk1 **
NM_022958	Phosphoinositide-3 class 3	Pik3c3 ****
NM_080688	Phospholipase C, delta 4	Plcd4 **
NM_133307	Protein Kinase C, delta	Prkcd *
AA799421	Protein Kinase C, epsilon	Prkce ***
BF284699	Protein Kinase C, iota	Prkci *
Table 3B LE gene expression > SD gene expression		
Accession #	Gene	Symbol
AW525609	Inositol(myo)-1(or 4)-monophosphatase 2	Impa2 *
BG380493	Multiple inositol polyphosphate histidine phosphatase, 1	Minpp1 *
D64048	Phosphoinositide–3-kinase, regulatory	Pik3r1 *****
AI102103	Phosphotidyl inositol, 4-kinase, catalytic beta	Pik4cb *
AI171093	Protein Kinase C, theta	Prkcq *

* $p < 0.01$

** $p < 0.001$

*** $p < 0.0001$

**** $p < 10^{-10}$

***** $p < 10^{-13}$

Table 4

Significant correlations between gene expression and APO PPI sensitivity in SD (A) and LE (B) rats

Expression values for genes in the following pathways: DA receptor signaling[1], synaptic long-term potentiation[2], and inositol phosphate metabolism[3] that exhibited significant between strain expression differences were correlated with % PPI at ISIs of 10 ms, 20 ms, and 120 ms. The most robust differences in APO-induced % PPI between SD and LE rats were detected at these ISIs. Significant Spearman correlations (R Value): all $p < 0.05$, or ** $p < 0.01$.

Table 4A		
Symbol	**Gene**	**R Value**
		ISI 10
Grin2b	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B [2]	0.713**
Prkci	Protein kinase C, iota [2,3]	-0.622
		ISI 20
Gria1	Glutamate receptor, ionotropic, AMPA1 [2]	-0.643
Prkcd	Protein kinase C, delta [2,3]	-0.587
		ISI 120
Adcy4	Adenylate cyclase 4 [1]	0.699
Inpp1	Inositol polyphosphate-1-phosphatase [3]	0.608
Table 4B		
Symbol	Gene	R Value
		ISI 10
Grm5	Glutamate receptor, metabotropic 5 [2]	0.682
		ISI 20
Adcy4	Adenylate cyclase 4 [1]	0.800**
Dgki	Diacylglycerol kinase, iota [2]	-0.682
		ISI 120
Ccr5	Ccr5 chemokine R5 [3]	0.800**
Inppl1	Inositol polyphosphate-1-phosphatase like [3]	-0.679