Published in final edited form as:

Schizophr Res. 2014 October ; 159(1): 130–135. doi:10.1016/j.schres.2014.07.040.

Increased G Protein-Coupled Receptor Kinase (GRK) Expression in the Anterior Cingulate Cortex in Schizophrenia

Adam J. Funk^a, Vahram Haroutunian^c, James H. Meador-Woodruff^{b,d}, and Robert E. McCullumsmith^a

^aDepartment of Psychiatry and Behavioral Neuroscience, University of Cincinnati, CARE 5830, 231 Albert Sabin Way, Cincinnati, OH 45267-0583

^bDepartment of Psychiatry and Behavioral Neurobiology, University of Alabama Birmingham, SC 560, 1530 3rd Avenue South, Birmingham, AL 35294

^cDepartment of Psychiatry, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1230, New York, NY 10029

^dEvelyn F. McKnight Brain Institute, University of Alabama Birmingham, Shelby 911, 1530 3rd Avenue South, Birmingham, AL 35294

Abstract

Background—Current pharmacological treatments for schizophrenia target G protein-coupled receptors (GPCRs), including dopamine receptors. Ligand bound GPCRs are regulated by a family of G protein-coupled receptor kinases (GRKs), members of which uncouple the receptor from heterotrimeric G proteins, desensitize the receptor, and induce receptor internalization via the arrestin family of scaffolding and signaling molecules. GRKs initiate the activation of downstream signaling pathways, can regulate receptors and signaling molecules independent of GPCR phosphorylation, and modulate epigenetic regulators like histone deacetylases (HDACs). We hypothesize that expression of GRK proteins are altered in schizophrenia, consistent with previous findings of alterations up and downstream from this family of molecules that facilitate intracellular signaling processes.

Methods—In this study we measured protein expression via Western blot analysis for GRKs 2, 3, 5, and 6 in the anterior cingulate cortex of patients with schizophrenia (N = 36) and a

© 2014 Elsevier B.V. All rights reserved

Address all correspondence to: Robert E. McCullumsmith, MD, PhD University of Cincinnati CARE 5830 231 Albert Sabin Way Cincinnati, OH 45267-0583 Department of Psychiatry and Behavioral Neuroscience Phone: 513-558-4855

robert.mccullumsmith@uc.edu.

(a) Current address

(b) Location of all experiments

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Contributions: Adam J Funk planned and performed the experiments with input from James Meador-Woodruff and Robert McCullumsmith. Vahram Haroutunian provided samples from the Mt. Sinai Brain Collection which he administers.

Conflict of Interest: The authors have nothing to disclose.

comparison group (N = 33). To control for antipsychotic treatment we measured these same targets in haloperidol treated vs. untreated rats (N = 10 for both).

Results—We found increased levels of GRK5 in schizophrenia. No changes were detected in GRK protein expression in rats treated with haloperidol decanoate for 9 months.

Conclusion—These data suggest that increased GRK5 expression may contribute to the pathophysiology of schizophrenia via abnormal regulation of the cytoskeleton, endocytosis, signaling, GPCRs, and histone modification.

Keywords

Schizophrenia; postmortem; G protein-coupled receptor kinase (GRK); G protein-coupled receptor (GPCR); kinase; histone deacetylase (HDAC)

1.1 INTRODUCTION

Converging hypotheses of the pathophysiology of schizophrenia have focused on G protein-coupled receptor (GPCR) dysfunction. GPCRs include a large, diverse population of receptors coupled to heterotrimeric G proteins, which transduce extracellular stimuli onto intracellular signaling pathways, and represent approximately half of all therapeutic drug targets (Bridges and Lindsley, 2008). Many GPCRs have been implicated in the pathophysiology of schizophrenia, including serotonin, dopamine, adrenergic, and metabotropic glutamate receptors. These receptors are regulated by multiple mechanisms, including desensitization and receptor internalization initiated by phosphorylation near the C-terminus of intracellular loops by members of the family of regulatory G protein-coupled receptor kinases (GRKs) (Beaulieu and Gainetdinov, 2011). There are seven GRKs identified to date that are grouped into three subgroups based on functional and sequence similarity. GRK1 and GRK7 phosphorylate rhodopsin and iodopsin, respectively, and are restricted to the retina. GRK2 and GRK3 (GRK2-like), and GRK4, GRK5, and GRK6 (GRK4-like), are expressed throughout the body and may regulate various types of GPCRs (Kelly et al., 2008).

GRK-induced GPCR phosphorylation initiates the recruitment of arrestins, a family of scaffolding, trafficking, and signaling proteins (Dromey and Pflieger, 2008). Arrestins facilitate GPCR internalization via clathrin coated pits, desensitization, and reinsertion in the plasma membrane (Kelly et al., 2008; Mundell et al., 2006; Premont and Gainetdinov, 2007). GRKs initiate the activation of many intracellular signaling molecules, including MAPKs and AKT, independent of GPCR kinase function. GRK5 and GRK6 contain nuclear localization sequences which facilitate translocation to the nucleus (Gurevich et al., 2012). Nuclear GRK5 associates with and phosphorylates HDAC5 (a class II histone deacetylase), initiating nuclear export (Chawla et al., 2003; Parra and Verdin, 2010). HDAC5 represses BDNF expression, and pharmacological inhibition of class II HDACs upregulates BDNF mRNA (Koppel and Timmusk, 2013). Interestingly, decreased BDNF expression is a consistent finding in schizophrenia, suggesting that studies examining regulators of BDNF are high yield targets for further investigation (Angelucci et al., 2005; Nurjono et al., 2012).

The anterior cingulate cortex (ACC) is an integration hub for error processing, associative pleasure vs pain, and higher order executive processing (Descalzi et al., 2009; Gao et al., 2009; Gasquoine, 2013; Shyu and Vogt, 2009; Toyoda et al., 2007). Neuroimaging studies consistently show abnormal ACC activation and microarray analyses from this cohort identified this region as one with high levels of altered transcript expression, making it an ideal region to study pathways and molecules which affect cognition (Gasquoine, 2013; Katsel et al., 2005).

Since GRKs are located synaptically, regulate GPCRs, modulate HDACs, and activate intracellular signaling pathways implicated in schizophrenia, we examined the hypothesis that GRKs are abnormally expressed in this illness. Using Western blot analysis, we measured the protein expression of GRKs 2,3,5,6 in the ACC, Brodmann area 32 (BA 32) from subjects with schizophrenia and a comparison group.

2.1 EXPERIMENTAL/MATERIALS AND METHODS

2.1.1 Postmortem brain tissue

Samples from the ACC (BA32) were obtained from the Mount Sinai Medical Center brain collection. Patients were diagnosed with schizophrenia using DSM-III-R criteria (Bauer et al., 2008). Each patient had a documented history of psychotic symptoms prior to the age of 40, and at least 10 years of hospitalization, with a diagnosis of schizophrenia made by two clinicians. Patients were recruited prospectively and underwent extensive antemortem diagnostic and clinical assessment. Subjects were excluded if they had a history of substance abuse, death by suicide, or coma for more than 6 hours before death. Neuropathological examination revealed no neurodegenerative diseases including Alzheimer's disease in any subjects. Next of kin consent was obtained for each patient (Bauer et al., 2008; Bauer et al., 2010; Bauer et al., 2009; Funk et al., 2009; Hammond et al., 2010; Oni-Orisan et al., 2008). Comparison subjects were selected using a formal blinded medical chart review instrument as well as in person interviews with the patients and/or their caregivers. Comparison subjects were also evaluated for, and were free of, dementia and neurodegenerative diseases, as well as any history of psychiatric illness or drug and alcohol abuse (Oni-Orisan et al., 2008). Schizophrenia and comparison groups were matched for sex, age, pH and PMI (Table 1). ACC RNA integrity (RIN) values were available for a majority of the subjects in this cohort. Control subjects had a median RIN of 5.37 with a standard deviation of 1.98, and Schizophrenia subjects had a median RIN of 4.97 with a standard deviation of 1.91

2.1.2 Protein sample preparation

Samples were obtained at autopsy from the left hemisphere. For protein studies, the ACC was dissected at the level of the genu of the corpus callosum. Gray matter was dissected from the white, and then gray matter samples were portioned into 1cm³ pieces and stored at -80°C until further processing. Tissue was pulverized into a powder using a mortar and pestle with a small amount of liquid nitrogen and stored at -80°C. Samples were reconstituted and homogenized in 5mM Tris-HCl pH 7.4, 0.32M sucrose and a protease inhibitor tablet (Complete Mini, Roche Diagnostics, Mannheim Germany) using a Power Gen 125 homogenizer (Thermo Fisher Scientific, Rockford, Illinois, USA) at speed 5 for 60

seconds. The homogenates were assayed for protein concentration using a BCA protein assay kit (Thermo Scientific, Rockford, Illinois), and stored at -80°C .

2.1.3 Antipsychotic treated rats

Rodent experiments were carried out according to University of Alabama-Birmingham (UAB) guidelines and all procedures complied with IACUC regulations. Male Sprague-Dawley rats (250g) were treated with haloperidol decanoate for 9 months as previously described (Drummond et al., 2012; Drummond et al., 2013). Briefly, rats were housed in pairs and injected intramuscularly every three weeks, for a total of 12 injections, with vehicle (sesame oil) or 28.5mg/kg of haloperidol decanoate in sesame oil. The dose of haloperidol decanoate is consistent with previous rodent studies (Harte et al., 2005; Kashihara et al., 1987; Kashihara et al., 1986; Mithani et al., 1987; Mithani et al., 1986). Rats were sacrificed by decapitation and the brains were immediately removed, dissected on wet ice, and the left anterior cortex was stored at -80°C . The tissue was prepared for Western blot analyses. Ten haloperidol decanoate treated and ten control rats were used for each experiment.

2.1.4 Western blot analysis

Human and rat experiments were performed as follows. The antisera outlined below were used in both human and rat experiments after test serial dilutions indicated linear detection using the protein amounts described. Samples for Western blot analysis were diluted with ultrapure (Milli-Q A10, Millipore) water and reducing buffer (6x solution: 4.5% SDS, 15% β -mercaptoethanol, 0.018% Bromophenol blue and 36% glycerol in 170mM Tris-HCl pH 6.8) to a concentration of 20 μg of protein per 12 μl and heated at 70°C for 10 minutes. Samples were then processed in duplicate by SDS-PAGE using Invitrogen (Carlsbad, California) 4–12% gradient gels and transferred to PVDF membranes via BioRad semi-dry transblotters (Hercules, California). The membranes were blocked with LiCor blocking buffer (Lincoln, Nebraska) for 1 hour at room temperature, then probed with primary antisera (GRK2 predicted molecular weight of $\sim 80\text{kDa}$ (1:500, # ab32558 abcam, Cambridge, MA), GRK3 predicted molecular weight of $\sim 80\text{kDa}$ (1:1000, # ab104508 abcam), GRK5 predicted molecular weight of $\sim 68\text{kDa}$ (1:1000, # ab64943 abcam), and GRK6 predicted molecular weight of $\sim 66\text{kDa}$ (1:1000, # ab64915 abcam) diluted in 0.1% Tween LiCor blocking buffer). The membranes were washed twice for 10 minutes each in 0.1% Tween phosphate buffer solution (PBST) then probed with goat anti-mouse or goat anti-rabbit IR-Dye 670 or 800cw labeled secondary antisera (LiCor) in 0.1% Tween, 0.01% sodium dodecyl sulfate (SDS) LiCor blocking buffer for 1 hour at room temperature. Washes were repeated after secondary labeling, washing twice for 10 minutes in PBST then placed in water.

Membranes were imaged using a LiCor Odyssey scanner. Boxes were manually placed around each band of interest which returned near-infrared fluorescent values of raw intensity with intra-lane background subtracted using Odyssey 3.0 analytical software (LiCor, Lincoln, Nebraska).

The near-infrared fluorescence value for each protein of interest was normalized to the in-lane value of valosin containing protein (VCP) and this normalized ratio from duplicate lanes was averaged (Bauer et al., 2009; Funk et al., 2012). We found no changes in raw intensity values for VCP between the schizophrenia and comparison groups, consistent with previous reports (Bauer et al., 2009; Funk et al., 2009; Hammond et al., 2010).

Each GRK isoform antibody was tested for specificity prior to the experiments. Results from test Western blots gave a prominent band at the predicted molecular weight for each isoform based on antibody data sheets. Representative bands from our Western blots are consistent with previous reports from the literature (Ahmed et al., 2008a; Bezard et al., 2005; Bychkov et al., 2011a; Bychkov et al., 2012; Bychkov et al., 2011c; Bychkov et al., 2008a; Ertley et al., 2007; Salim and Eikenburg, 2007; Zhang et al., 2014).

2.1.5 Data analysis

Data were analyzed using Statistica (Statsoft, Tulsa, Oklahoma). All dependent measures were determined to have a Gaussian distribution. Correlation analyses were performed to determine associations between the dependent variables and pH, age and PMI. Sex and antipsychotic medication status were used as grouping variables for secondary analyses as previously described (Funk et al., 2012). T-tests were used to independently analyze each GRK isoform from both human and rat data. The effect of covariates was analyzed by ANCOVA, where applicable.

3.1 RESULTS

No significant associations between age, pH, or PMI and our dependent measures were detected other than GRK6 and pH ($R^2 = 0.077$, $(F(1, 65) = 5.42, p = 0.023)$). The resulting ANCOVA for GRK6 with diagnosis as the categorical predictor and pH as the continuous predictor showed no change for GRK6 protein expression ($F(1,64) = 1.33, p = 0.25$).

Using Western blot analysis, we found increased GRK5 ($t(1, 67) = 2.64, p = .01$) protein levels in schizophrenia (Figure 1). A report from the literature indicates that the lower (major) band may represent a phosphorylated form of GRK5 (Bychkov et al., 2012). For GRK5, both bands were included in the analysis. No changes were detected for GRK2 ($t(1, 67) = 1.437, p = 0.155$) or GRK3 ($t(1, 64) = 0.13, p = 0.896$). Only the major band at the correct molecular weight was analyzed for GRK2 and GRK3. Outlier analyses revealed the same two schizophrenia subjects for GRK2 and GRK5, the removal of which did not affect the statistical outcome for either protein (GRK2 ($t(1,65) = 0.6511, p = 0.52$) and GRK5 ($t(1,65) = 2.45, p = 0.017$)).

In rats treated with haloperidol decanoate for 9 months, we detected no changes in GRK2, GRK3, GRK5, or GRK6 protein expression (Figure 2). We were unable to detect GRK4 protein levels in either human or rat samples in this region, consistent with previous reports (Premont et al., 1995; Premont et al., 1999; Virlon et al., 1998). Analyses among the schizophrenia group based on medication status were performed to determine any changes between subjects on vs off antipsychotics for six weeks or more at time of death. The data

indicate no change for any GRK isoform, GRK2 ($F(1,34) = 0.98$, $p = 0.33$, GRK3 ($F(1,32) = 3.54$, $p = 0.07$, GRK5 ($F(1,34) = 0.33$, $p = 0.57$, and GRK6 ($F(1,33) = 0.95$, $p = 0.34$).

4.1 DISCUSSION

In this study, we found increased GRK5 protein expression in the ACC in schizophrenia. GRK5 is ubiquitously expressed in the brain and modulates ligand bound GPCR desensitization and internalization (Erdtmann-Vourliotis et al., 2001). These results suggest relative uncoupling of GPCRs from their ability to propagate intracellular signals in schizophrenia. These data add to the literature of alterations in regulatory and downstream signaling pathways in this illness (Baracskey et al., 2006; Clinton et al., 2005; Emamian et al., 2004; Kyosseva, 2004a, b; Kyosseva et al., 1999; Manji et al., 2003; Todorova et al., 2003; Yuan et al., 2010).

GRK5 is implicated in multiple developmental and degenerative CNS disorders. For example, α -synuclein is phosphorylated by GRK5, and GRK5 mRNA is increased in prodromal and early Alzheimer's patients (Suo and Li, 2010), while levels of GRK5 are positively correlated with severity of dementia in Alzheimer's disease (AD) (Suo and Li, 2010). Patients with Parkinson's disease with dementia express higher amounts of GRK5 in the striatum than non-demented patients (Bychkov et al., 2008b). Taken together these data suggest that changes in GRK5 are associated with the pathophysiology of severe neuropsychiatric illnesses associated with cognitive impairment. GRK5 abnormalities are also associated with cocaine administration, major depressive disorder, as well as behavioral hypersensitivity, consistent with the central role(s) of this molecule for linking GPCR activation with intracellular signaling processes (Bychkov et al., 2011a; Erdtmann-Vourliotis et al., 2001; Franklin and Carrasco, 2013; Gainetdinov et al., 1999; Ji et al., 2013; Kara et al., 2006; Luo et al., 2008; Ren et al., 2005; Shenoy et al., 2006; Zheng et al., 2012; Zhu et al., 2004; Zhu et al., 2013).

A role for GRK5 has been postulated in schizophrenia. In a neonatal ventral hippocampal lesion model of schizophrenia, GRK5 expression was reduced in prefrontal cortex of female versus male rats (Bychkov et al., 2011b). Another study from the same group comprehensively assessed GRKs, arrestins, and expression of other kinases in the prefrontal cortex (Bychkov et al., 2011c). This study utilized tissues from 3 different brain collections, including the Bronx VA/Mt. Sinai brain bank which was the source of the tissues for our study. Using data grouped from all 3 cohorts, decreased GRK3 and a trend for lower GRK2 and GRK5 protein expression was found in the DLPFC, while decreased GRK6 was found in the same region using only the Bronx VA/Mt. Sinai samples. The authors also found an inverse correlation between age and expression of several GRK proteins, as well as decreases in GRK2 and GRK3 in specific subcellular fractions (Ahmed et al., 2008a). They did not examine the ACC in this study. These data support the hypothesis that GRK abnormalities may contribute to the pathophysiology of schizophrenia, and indicate that GRK abnormalities may be region specific.

A potential limitation of our study is lifelong antipsychotic treatment of subjects with schizophrenia, since dopamine D1 and D2 receptors can be regulated by GRK5, and typical

and atypical antipsychotics may modulate its expression (Ahmed et al., 2008b; Tiberi et al., 1996). We addressed this potential confound in two ways. We analyzed schizophrenia subjects on versus off antipsychotic medication for at least 6 weeks and detected no differences for any of our dependent measures. We also examined GRK protein expression in the frontal cortex of rats treated for 9 months with haloperidoldecanoate, and found no differences. Taken together, these data suggest that our finding of altered GRK5 protein expression is not due to a medication effect.

These changes in diverse illnesses may be mediated via GRK5 modulation of HDAC expression, trafficking, and function. Increased GRK5 expression would suggest altered interactome regulation, including HDAC5 (Chawla et al., 2003; Parra and Verdin, 2010). Multiple lines of evidence support the hypothesis of HDAC5 dysregulation conferring liability to neurological illness. For example, reduction of HDAC5 causes impaired learning and memory in a model of AD (Agis-Balboa et al., 2013) and there is dysregulation of HDAC5 mRNA in premutation carriers of Fragile X (Mateu-Huertas et al., 2014). Chronic social defeat reduces BDNF expression, which is rescued by administration of imipramine and an associated downregulation of HDAC5 (Tsankova et al., 2006). Thus, an abnormality in GRK5 expression may act via multiple mechanisms, including altered HDAC5 regulation in schizophrenia.

We have previously reported abnormal mGluR expression in schizophrenia, including increased mGluR1a and mGluR2/3 protein expression levels in prefrontal cortex (Gupta et al., 2005). Interestingly, GRK2 desensitizes agonist bound mGluR3, inhibiting the ability of mGluR3 to regulate the generation of cAMP from ATP via adenylyl cyclase (Iacovelli et al., 2009). GRK2 also inhibits mGluR2 and mGluR3 initiated activation of the MAPK pathway, independent of cAMP regulation (Iacovelli et al., 2009). Increased protein expression of mGluR2/3 in the prefrontal cortex in schizophrenia, with no concurrent increase in GRK2 or 3 protein expression, may then be associated with decreased downstream signal activation in this illness.

GRKs are enzymes critical for the regulation of GPCRs, regulate signaling pathways and histone modification, and have been previously implicated in neuropsychiatric disorders. This study provides additional evidence for their involvement in schizophrenia. These data add to a growing literature of receptor regulatory and signal integration abnormalities in schizophrenia, which may point to new therapeutic targets for this psychiatric illness.

Acknowledgments

The authors would like to thank the patients and their families for their kind donations of postmortem brain tissue.

Funding: This work was supported by grants MH53327 (JMW), MH064673 & MH066392 (VH), MH094445, MH074016 and Doris Duke Clinical Scientist Award (REM)

References

Agis-Balboa RC, Pavelka Z, Kerimoglu C, Fischer A. Loss of HDAC5 impairs memory function: implications for Alzheimer's disease. *Journal of Alzheimer's disease : JAD*. 2013; 33(1):35–44.

- Ahmed MR, Bychkov E, Gurevich VV, Benovic JL, Gurevich EV. Altered expression and subcellular distribution of GRK subtypes in the dopamine-depleted rat basal ganglia is not normalized by L-DOPA treatment. *Journal of neurochemistry*. 2008a; 104(6):1622–1636. [PubMed: 17996024]
- Ahmed MR, Gurevich VV, Dalby KN, Benovic JL, Gurevich EV. Haloperidol and clozapine differentially affect the expression of arrestins, receptor kinases, and extracellular signal-regulated kinase activation. *The Journal of pharmacology and experimental therapeutics*. 2008b; 325(1):276–283. [PubMed: 18178904]
- Angelucci F, Brene S, Mathe AA. BDNF in schizophrenia, depression and corresponding animal models. *Molecular psychiatry*. 2005; 10(4):345–352. [PubMed: 15655562]
- Baracska K, Haroutunian V, Meador-Woodruff J. Dopamine receptor signaling molecules are altered in elderly schizophrenic cortex. *Synapse*. 2006; 60(4):271–279. [PubMed: 16786528]
- Bauer D, Gupta D, Haroutunian V, Meador-Woodruff J, McCullumsmith R. Abnormal expression of glutamate transporter and transporter interacting molecules in prefrontal cortex in elderly patients with schizophrenia. *Schizophr Res*. 2008; 104(1–3):108–120. [PubMed: 18678470]
- Bauer D, Haroutunian V, Meador-Woodruff J, McCullumsmith R. Abnormal glycosylation of EAAT1 and EAAT2 in prefrontal cortex of elderly patients with schizophrenia. *Schizophr Res*. 2010; 117(1):92–98. [PubMed: 19716271]
- Bauer DE, Haroutunian V, McCullumsmith RE, Meador-Woodruff JH. Expression of four housekeeping proteins in elderly patients with schizophrenia. *Journal of neural transmission*. 2009; 116(4):487–491. [PubMed: 19139805]
- Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological reviews*. 2011; 63(1):182–217. [PubMed: 21303898]
- Bezard E, Gross CE, Qin L, Gurevich VV, Benovic JL, Gurevich EV. L-DOPA reverses the MPTP-induced elevation of the arrestin2 and GRK6 expression and enhanced ERK activation in monkey brain. *Neurobiology of disease*. 2005; 18(2):323–335. [PubMed: 15686961]
- Bridges TM, Lindsley CW. G-protein-coupled receptors: from classical modes of modulation to allosteric mechanisms. *ACS Chem Biol*. 2008; 3(9):530–541. [PubMed: 18652471]
- Bychkov E, Ahmed MR, Gurevich EV. Sex differences in the activity of signalling pathways and expression of G-protein-coupled receptor kinases in the neonatal ventral hippocampal lesion model of schizophrenia. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*. 2011a; 14(1):1–15.
- Bychkov E, Ahmed MR, Gurevich EV. Sex differences in the activity of signalling pathways and expression of G-protein-coupled receptor kinases in the neonatal ventral hippocampal lesion model of schizophrenia. *Int J Neuropsychopharmacol*. 2011b; 14(1):1–15. [PubMed: 20158934]
- Bychkov E, Zurkovsky L, Garret MB, Ahmed MR, Gurevich EV. Distinct cellular and subcellular distributions of G protein-coupled receptor kinase and arrestin isoforms in the striatum. *PloS one*. 2012; 7(11):e48912. [PubMed: 23139825]
- Bychkov ER, Ahmed MR, Gurevich VV, Benovic JL, Gurevich EV. Reduced expression of G protein-coupled receptor kinases in schizophrenia but not in schizoaffective disorder. *Neurobiology of disease*. 2011c; 44(2):248–258. [PubMed: 21784156]
- Bychkov ER, Gurevich VV, Joyce JN, Benovic JL, Gurevich EV. Arrestins and two receptor kinases are upregulated in Parkinson's disease with dementia. *Neurobiology of aging*. 2008a; 29(3):379–396. [PubMed: 17125886]
- Bychkov ER, Gurevich VV, Joyce JN, Benovic JL, Gurevich EV. Arrestins and two receptor kinases are upregulated in Parkinson's disease with dementia. *Neurobiol Aging*. 2008b; 29(3):379–396. [PubMed: 17125886]
- Chawla S, Vanhoutte P, Arnold FJ, Huang CL, Bading H. Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. *Journal of neurochemistry*. 2003; 85(1):151–159. [PubMed: 12641737]
- Clinton SM, Ibrahim HM, Frey KA, Davis KL, Haroutunian V, Meador-Woodruff JH. Dopaminergic abnormalities in select thalamic nuclei in schizophrenia: involvement of the intracellular signal integrating proteins calcyon and spinophilin. *Am J Psychiatry*. 2005; 162(10):1859–1871. [PubMed: 16199832]

- Descalzi G, Kim S, Zhuo M. Presynaptic and postsynaptic cortical mechanisms of chronic pain. *Mol Neurobiol.* 2009; 40(3):253–259. [PubMed: 19821080]
- Dromey JR, Pflieger KD. G protein coupled receptors as drug targets: the role of beta-arrestins. *Endocr Metab Immune Disord Drug Targets.* 2008; 8(1):51–61. [PubMed: 18393923]
- Drummond JB, Simmons M, Haroutunian V, Meador-Woodruff JH. Upregulation of cornichon transcripts in the dorsolateral prefrontal cortex in schizophrenia. *Neuroreport.* 2012; 23(17):1031–1034. [PubMed: 23103966]
- Drummond JB, Tucholski J, Haroutunian V, Meador-Woodruff JH. Transmembrane AMPA receptor regulatory protein (TARP) dysregulation in anterior cingulate cortex in schizophrenia. *Schizophrenia research.* 2013; 147(1):32–38. [PubMed: 23566497]
- Emamian E, Karayiorgou M, Gogos J. Decreased phosphorylation of NMDA receptor type 1 at serine 897 in brains of patients with Schizophrenia. *J Neurosci.* 2004; 24(7):1561–1564. [PubMed: 14973229]
- Erdtmann-Vourliotis M, Mayer P, Ammon S, Riechert U, Holtt V. Distribution of G-protein-coupled receptor kinase (GRK) isoforms 2, 3, 5 and 6 mRNA in the rat brain. *Brain research. Molecular brain research.* 2001; 95(1–2):129–137. [PubMed: 11687284]
- Ertley RN, Bazinet RP, Lee HJ, Rapoport SI, Rao JS. Chronic treatment with mood stabilizers increases membrane GRK3 in rat frontal cortex. *Biological psychiatry.* 2007; 61(2):246–249. [PubMed: 16697355]
- Franklin JM, Carrasco GA. G-protein receptor kinase 5 regulates the cannabinoid receptor 2-induced up-regulation of serotonin 2A receptors. *The Journal of biological chemistry.* 2013; 288(22):15712–15724. [PubMed: 23592773]
- Funk A, Rumbaugh G, Haroutunian V, McCullumsmith R, Meador-Woodruff J. Decreased expression of NMDA receptor-associated proteins in frontal cortex of elderly patients with schizophrenia. *Neuroreport.* 2009; 20(11):1019–1022. [PubMed: 19483657]
- Funk AJ, McCullumsmith RE, Haroutunian V, Meador-Woodruff JH. Abnormal activity of the MAPK- and cAMP-associated signaling pathways in frontal cortical areas in postmortem brain in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2012; 37(4):896–905. [PubMed: 22048463]
- Gainetdinov RR, Bohn LM, Walker JK, Laporte SA, Macrae AD, Caron MG, Lefkowitz RJ, Premont RT. Muscarinic supersensitivity and impaired receptor desensitization in G protein-coupled receptor kinase 5-deficient mice. *Neuron.* 1999; 24(4):1029–1036. [PubMed: 10624964]
- Gao Y, Zhang L, Samad O, Suter M, Yasuhiko K, Xu Z, Park J, Lind A, Ma Q, Ji R. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. *J Neurosci.* 2009; 29(13):4096–4108. [PubMed: 19339605]
- Gasquoine PG. Localization of function in anterior cingulate cortex: from psychosurgery to functional neuroimaging. *Neuroscience and biobehavioral reviews.* 2013; 37(3):340–348. [PubMed: 23313645]
- Gupta D, McCullumsmith R, Beneyto M, Haroutunian V, Davis K, Meador-Woodruff J. Metabotropic glutamate receptor protein expression in the prefrontal cortex and striatum in schizophrenia. *Synapse.* 2005; 57(3):123–131. [PubMed: 15945063]
- Gurevich EV, Tesmer JJ, Mushegian A, Gurevich VV. G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. *Pharmacology & therapeutics.* 2012; 133(1):40–69. [PubMed: 21903131]
- Hammond J, McCullumsmith R, Funk A, Haroutunian V, Meador-Woodruff J. Evidence for abnormal forward trafficking of AMPA receptors in frontal cortex of elderly patients with schizophrenia. *Neuropsychopharmacology.* 2010; 35(10):2110–2119. [PubMed: 20571483]
- Harte MK, Bachus SB, Reynolds GP. Increased N-acetylaspartate in rat striatum following long-term administration of haloperidol. *Schizophrenia research.* 2005; 75(2–3):303–308. [PubMed: 15885521]
- Iacovelli L, Molinaro G, Battaglia G, Motolese M, Di Menna L, Alfiero M, Blahos J, Matrisciano F, Corsi M, Corti C, Bruno V, De Blasi A, Nicoletti F. Regulation of group II metabotropic glutamate receptors by G protein-coupled receptor kinases: mGlu2 receptors are resistant to homologous desensitization. *Mol Pharmacol.* 2009; 75(4):991–1003. [PubMed: 19164443]

- Ji Y, Biernacka JM, Hebbring S, Chai Y, Jenkins GD, Batzler A, Snyder KA, Drews MS, Desta Z, Flockhart D, Mushiroda T, Kubo M, Nakamura Y, Kamatani N, Schaid D, Weinshilboum RM, Mrazek DA. Pharmacogenomics of selective serotonin reuptake inhibitor treatment for major depressive disorder: genome-wide associations and functional genomics. *The pharmacogenomics journal*. 2013; 13(5):456–463. [PubMed: 22907730]
- Kara E, Crepieux P, Gauthier C, Martinat N, Piketty V, Guillou F, Reiter E. A phosphorylation cluster of five serine and threonine residues in the C-terminus of the follicle-stimulating hormone receptor is important for desensitization but not for beta-arrestin-mediated ERK activation. *Molecular endocrinology*. 2006; 20(11):3014–3026. [PubMed: 16887887]
- Kashihara K, Fukuda K, Sato M, Otsuki S. Haloperidol prevents the methamphetamine-induced apomorphine subsensitivity of dopamine metabolism in rat striatum. *Neuroscience research*. 1987; 4(5):428–432. [PubMed: 3670749]
- Kashihara K, Sato M, Fujiwara Y, Harada T, Ogawa T, Otsuki S. Effects of intermittent and continuous haloperidol administration on the dopaminergic system in the rat brain. *Biological psychiatry*. 1986; 21(7):650–656. [PubMed: 3011131]
- Katsel P, Davis KL, Gorman JM, Haroutunian V. Variations in differential gene expression patterns across multiple brain regions in schizophrenia. *Schizophr Res*. 2005; 77(2–3):241–252. [PubMed: 15923110]
- Kelly E, Bailey CP, Henderson G. Agonist-selective mechanisms of GPCR desensitization. *Br J Pharmacol*. 2008; 153(Suppl 1):S379–388. [PubMed: 18059321]
- Koppel I, Timmusk T. Differential regulation of Bdnf expression in cortical neurons by class-selective histone deacetylase inhibitors. *Neuropharmacology*. 2013; 75:106–115. [PubMed: 23916482]
- Kyosseva S. Differential expression of mitogen-activated protein kinases and immediate early genes fos and jun in thalamus in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004a; 28(6):997–1006. [PubMed: 15380860]
- Kyosseva S. The role of the extracellular signal-regulated kinase pathway in cerebellar abnormalities in schizophrenia. *Cerebellum*. 2004b; 3(2):94–99. [PubMed: 15233576]
- Kyosseva S, Elbein A, Griffin W, Mrak R, Lyon M, Karson C. Mitogen-activated protein kinases in schizophrenia. *Biol Psychiatry*. 1999; 46(5):689–696. [PubMed: 10472421]
- Luo J, Busillo JM, Benovic JL. M3 muscarinic acetylcholine receptor-mediated signaling is regulated by distinct mechanisms. *Molecular pharmacology*. 2008; 74(2):338–347. [PubMed: 18388243]
- Manji HK, Gottesman II, Gould TD. Signal transduction and genes-to-behaviors pathways in psychiatric diseases. *Sci STKE*. 2003; 2003(207):pe49. [PubMed: 14600293]
- Mateu-Huertas E, Rodriguez-Reventa L, Alvarez-Mora MI, Madrigal I, Willemsen R, Mila M, Marti E, Estivill X. Blood expression profiles of fragile X premutation carriers identify candidate genes involved in neurodegenerative and infertility phenotypes. *Neurobiology of disease*. 2014; 65C:43–54. [PubMed: 24418349]
- Mithani S, Atmadja S, Baimbridge KG, Fibiger HC. Neuroleptic-induced oral dyskinesias: effects of progabide and lack of correlation with regional changes in glutamic acid decarboxylase and choline acetyltransferase activities. *Psychopharmacology*. 1987; 93(1):94–100. [PubMed: 2888156]
- Mithani S, Martin-Iverson MT, Phillips AG, Fibiger HC. The effects of haloperidol on amphetamine- and methylphenidate-induced conditioned place preferences and locomotor activity. *Psychopharmacology*. 1986; 90(2):247–252. [PubMed: 3097706]
- Mundell SJ, Luo J, Benovic JL, Conley PB, Poole AW. Distinct clathrin-coated pits sort different G protein-coupled receptor cargo. *Traffic*. 2006; 7(10):1420–1431. [PubMed: 16899088]
- Nurjono M, Lee J, Chong SA. A Review of Brain-derived Neurotrophic Factor as a Candidate Biomarker in Schizophrenia. *Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology*. 2012; 10(2):61–70. [PubMed: 23431036]
- Oni-Orisan A, Kristiansen L, Haroutunian V, Meador-Woodruff J, McCullumsmith R. Altered vesicular glutamate transporter expression in the anterior cingulate cortex in schizophrenia. *Biol Psychiatry*. 2008; 63(8):766–775. [PubMed: 18155679]

- Parra M, Verdin E. Regulatory signal transduction pathways for class IIa histone deacetylases. *Current opinion in pharmacology*. 2010; 10(4):454–460. [PubMed: 20447866]
- Premont RT, Gainetdinov RR. Physiological roles of G protein-coupled receptor kinases and arrestins. *Annu Rev Physiol*. 2007; 69:511–534. [PubMed: 17305472]
- Premont RT, Inglese J, Lefkowitz RJ. Protein kinases that phosphorylate activated G protein-coupled receptors. *FASEB J*. 1995; 9(2):175–182. [PubMed: 7781920]
- Premont RT, Macrae AD, Aparicio SA, Kendall HE, Welch JE, Lefkowitz RJ. The GRK4 subfamily of G protein-coupled receptor kinases. Alternative splicing, gene organization, and sequence conservation. *J Biol Chem*. 1999; 274(41):29381–29389. [PubMed: 10506199]
- Ren XR, Reiter E, Ahn S, Kim J, Chen W, Lefkowitz RJ. Different G protein-coupled receptor kinases govern G protein and beta-arrestin-mediated signaling of V2 vasopressin receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(5):1448–1453. [PubMed: 15671180]
- Salim S, Eikenburg DC. Role of 90-kDa heat shock protein (Hsp 90) and protein degradation in regulating neuronal levels of G protein-coupled receptor kinase 3. *The Journal of pharmacology and experimental therapeutics*. 2007; 320(3):1106–1112. [PubMed: 17179467]
- Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, Reiter E, Premont RT, Lichtarge O, Lefkowitz RJ. beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *The Journal of biological chemistry*. 2006; 281(2):1261–1273. [PubMed: 16280323]
- Shyu B, Vogt B. Short-term synaptic plasticity in the nociceptive thalamic-anterior cingulate pathway. *Mol Pain*. 2009; 5:51. [PubMed: 19732417]
- Suo WZ, Li L. Dysfunction of G protein-coupled receptor kinases in Alzheimer's disease. *ScientificWorldJournal*. 2010; 10:1667–1678. [PubMed: 20730384]
- Tiberi M, Nash SR, Bertrand L, Lefkowitz RJ, Caron MG. Differential regulation of dopamine D1A receptor responsiveness by various G protein-coupled receptor kinases. *The Journal of biological chemistry*. 1996; 271(7):3771–3778. [PubMed: 8631993]
- Todorova V, Elbein A, Kyosseva S. Increased expression of c-Jun transcription factor in cerebellar vermis of patients with schizophrenia. *Neuropsychopharmacology*. 2003; 28(8):1506–1514. [PubMed: 12799614]
- Toyoda H, Zhao M, Xu H, Wu L, Ren M, Zhuo M. Requirement of extracellular signal-regulated kinase/mitogen-activated protein kinase for long-term potentiation in adult mouse anterior cingulate cortex. *Mol Pain*. 2007; 3:36. [PubMed: 18053155]
- Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nature neuroscience*. 2006; 9(4):519–525.
- Virlon B, Firsov D, Cheval L, Reiter E, Troispoux C, Guillou F, Elalouf JM. Rat G protein-coupled receptor kinase GRK4: identification, functional expression, and differential tissue distribution of two splice variants. *Endocrinology*. 1998; 139(6):2784–2795. [PubMed: 9607785]
- Yuan P, Zhou R, Wang Y, Li X, Li J, Chen G, Guitart X, Manji HK. Altered levels of extracellular signal-regulated kinase signaling proteins in postmortem frontal cortex of individuals with mood disorders and schizophrenia. *J Affect Disord*. 2010; 124(1–2):164–169. [PubMed: 19913919]
- Zhang Y, Chen L, Shen G, Zhao Q, Shangguan L, He M. GRK5 dysfunction accelerates tau hyperphosphorylation in APP (swe) mice through impaired cholinergic activity. *Neuroreport*. 2014
- Zheng H, Worrall C, Shen H, Issad T, Seregard S, Girnita A, Girnita L. Selective recruitment of G protein-coupled receptor kinases (GRKs) controls signaling of the insulin-like growth factor 1 receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109(18):7055–7060. [PubMed: 22509025]
- Zhu M, Fan XL, Yang WL, Jiang Y, Ma L. Regulation of G protein-coupled receptor kinase 5 mRNA and protein level in rat brain by addictive drugs. *Sheng li xue bao : [Acta physiologica Sinica]*. 2004; 56(5):559–565.
- Zhu W, Tilley DG, Myers VD, Coleman RC, Feldman AM. Arginine vasopressin enhances cell survival via a G protein-coupled receptor kinase 2/beta-arrestin1/extracellular-regulated kinase

1/2-dependent pathway in H9c2 cells. *Molecular pharmacology*. 2013; 84(2):227–235. [PubMed: 23690069]

Figure 1.

GRK Protein Expression in Anterior Cingulate Cortex in Schizophrenia. Protein expression for GRK5 was increased in subjects with schizophrenia. GRK2, GRK3, and GRK6 protein levels did not differ between groups. Protein expression values were normalized to VCP as an in-lane loading control. * $p < 0.05$, C = comparison group, S = schizophrenia group.

Figure 2.

GRK Protein Expression in Anterior Cortex of Haloperidol Decanoate Treated Rats. Protein levels did not differ between rats treated with haloperidol decanoate for 9 months and vehicle. Protein expression values were normalized to VCP as an in-lane loading control. C = vehicle, H = haloperidol decanoate treated.

Table 1

Subject Characteristics

Category	Comparison	Schizophrenia
N	33	36
Sex	14m / 19f	24m / 12f
Tissue pH	6.4 ± 0.2	6.4 ± 0.3
PMI (hours)	8.3 ± 6.8	13.2 ± 8.0
Age (years)	78 ± 14	74 ± 11
On / Off Rx	0 / 33	25 / 11

Values presented as means ± standard deviation

Abbreviations: postmortem interval (PMI)

On Rx = number of patients taking antipsychotic medication at time of death

Off Rx = number of patients neuroleptic free at least 6 weeks prior to death