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BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Niswender, Colleen M.	Associate Professor of Pharmacology
eRA COMMONS USER NAME niswenc	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Akron		1986-1987	Biology
University of Toledo	B.S.	1987-1991	Pharmacy
Vanderbilt University	Ph.D.	1991-1996	Pharmacology
Vanderbilt University		1996-1998	Pharmacology
University of Washington		1998-2004	Pharmacology

A. Personal Statement

I am an Associate Professor of Pharmacology at Vanderbilt University Medical Center and am a highly trained molecular biologist and pharmacologist with a great deal of experience in assay development, high throughput screening and characterization of novel GPCR ligands, and RNA analysis. During my graduate training and initial postdoctoral studies, I worked to elucidate the molecular mechanisms regulating the RNA editing of AMPA and serotonin receptors. I then pursued postdoctoral studies on the genetic regulation of protein kinase A using recombinant mice at the University of Washington. In addition to my academic appointment, I am the Director of Molecular Pharmacology for the Vanderbilt Center for Neuroscience Drug Discovery and have been involved in small molecule discovery to search for and characterize novel allosteric modulators of various metabotropic glutamate and muscarinic receptors; my particular focus has been on the basic biology and pharmacological tool and drug development for the metabotropic glutamate receptors.

B. Positions and Honors

Employment

1996-1998 Postdoctoral Fellow, Vanderbilt University
1998-2003 Senior Fellow, Pharmacology, University of Washington
2003-2004 Acting Instructor, Pharmacology, University of Washington
2004-2009 Assistant Professor, Pharmacology, Co-Director, Molecular Pharmacology, Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University
2009-present Associate Professor, Pharmacology, Director, Molecular Pharmacology, Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University

Honors and Awards

1991 Valedictorian, College of Pharmacy
Phi Kappa Phi Graduate Fellowship
AAPS-AFPE Gateway Scholarship
The Merck-Sharp and Dohme Award in Medicinal Chemistry
The Upjohn Award in Pharmacology
1993 First Place, Vanderbilt University Graduate Student Research Day
1994 First Place, Vanderbilt University Graduate Student Research Day
1994-1996 Pharmaceutical Research and Manufacturers of American Foundation Predoctoral Award in Pharmacology
1997-1998 Pharmaceutical Research and Manufacturers of American Foundation Postdoctoral Award in Pharmacology
1999-2000 Fellow, Neurobiology and Behavior Training Grant, University of Washington
2000-2003 Fellow, National Service Research Award, NIDDK
2000 Travel award for abstract submitted to “Obesity and the Regulation of Energy Homeostasis”, Keystone Symposium, Taos, NM
2003-2004 DERC Pilot and Feasibility Award Recipient

Professional Memberships

1991-1998, 2006-present Society for Neuroscience
2008-present American Society of Pharmacology and Therapeutics

C. Contribution to Science

RNA editing within the mammalian central nervous system and implications for suicide

Adenosine-to-inosine RNA editing is mediated by a family of adenosine deaminases (ADARs) and has the potential to post-transcriptionally alter the amino acid codons within messenger RNA. An early example of this type of editing event is a conversion event within the AMPA GluR2 subunit, which changes a glutamine (CAG) to an arginine (CIG, read as "CGG" during translation). This alteration, which occurs within a region of the receptor subunit that lines the pore of the tetrameric AMPA receptor, exquisitely controls calcium conductance of AMPA receptors with edited subunits. During my graduate work, my co-workers and I showed that editing of the GluR2 subunit was specifically caused by A-to-I editing, and was consistent with an adenosine deaminase. In addition to my work on GluR2, I also showed that the RNA encoding the G protein-coupled serotonin 2C receptor (5-HT_{2C}R) was edited, resulting in 24 distinct protein isoforms. Editing of the 5-HT_{2C}R occurs within the second intracellular loop and alters the G protein coupled profile of the receptor. Our work showed that the fully edited 5-HT_{2C}R, containing the amino acids Val-Gly-Val (VGV) in place of the nonedited version, Ile-Asn-Ile (INI), exhibits drastically reduced levels of constitutive activity, a hallmark of the INI receptor version. Additional studies using human tissues showed that there were significant alterations in editing patterns in patients who had committed suicide. This work has now been substantiated by six other reports, all showing variations in editing patterns in patients who committed suicide. Overall, this body of work describes a highly relevant, post-transcriptional phenomenon that controls protein isoform expression with relevance to human psychiatric disorders. It should also be noted that the 5-HT_{2C}R remains the only GPCR identified to date that is subject to regulation by RNA editing.

1. *Rueter SM, ***Burns CM**, Coode SA, Mookherje P, Emeson RB. Glutamate receptor RNA editing in vitro by enzymatic conversion of adenosine to inosine. *Science* 1995;267:1491-1494. ***co-first authors; maiden name is Burns**
2. **Burns CM**, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emeson RB. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature* 1997;387:303-308.
3. **Niswender CM**, Copeland SC, Emeson RB, Sanders-Bush E. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *Journal of Biological Chemistry* 1999;274(14):9472-9478.
4. **Niswender CM,** Herrick-Davis K, Dilley GE, Meltzer HY, Overholser JC, Stockmeier CA, Emeson RB, Sanders-Bush, E. RNA editing of the human serotonin 5-HT2C receptor: alterations in suicide and implications for serotonergic pharmacotherapy. *Neuropsychopharmacology* 2001, 24(5):478-491.

Small molecule discovery and development of $mGlu_4$ positive allosteric modulators for the treatment of Parkinson's disease and other CNS disorders

The primary neuropathology of Parkinson's disease involves the progressive degeneration of dopamine neurons in the substantia nigra pars compacta (SNc), which provide the major dopaminergic innervation to the striatum and other basal ganglia (BG) nuclei. Within the BG, movement is controlled via a balance between two GABAergic pathways that project from the striatum either directly to the BG output nuclei (the direct pathway), or polysynaptically, projecting from the striatum to the globus pallidus, then to the substantia nigra, and then to the output nuclei (the indirect pathway). $mGlu_4$ is highly expressed presynaptically on terminals projecting from the striatum to the globus pallidus and activation of $mGlu_4$ decreases overactive GABA release from these projections, resulting in antiparkinsonian action. Over the past eight years, I have been the Biology Team Lead for a project focused on optimizing small molecule positive allosteric modulators (PAMs) of $mGlu_4$. These studies resulted in a successful Linked Effort to Accelerate Parkinson's Solutions (LEAPS) award from the Michael J. Fox Foundation and licensing of lead compounds and intellectual property to a major pharmaceutical company, Bristol Myers-Squibb. This work has now resulted in the filing of 14 patents

comprising composition of matter for $mGlu_4$ PAMs. In addition to my role as Biology Team Leader, I have served as the Director of Molecular Pharmacology for the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD). The VCNDD is a unique and collaborative environment that combines molecular pharmacology, medicinal chemistry, drug metabolism and pharmacokinetics, and *in vivo* pharmacology to de-risk early stage drug discovery. This has resulted in eight programs that have been licensed to various pharmaceutical partners, over 350 patents now held at Vanderbilt University, and over 330 publications from the Center.

1. **Niswender CM**, Myers KA, Kim, C, Ayala JE, Conn PJ, Weaver CD. Development of a novel and direct assay for high throughput screening of $G_{i/o}$-linked G protein coupled receptors using thallium flux through GIRK channels. *Molecular Pharmacology* 2008, 73(4):1213-1224.
2. **Niswender CM**, Johnson KA, Weaver CD, Jones CK, Xiang Z, Luo Q, Rodriguez AL, Marlo JE, de Paulis T, Thompson AD, Days EL, Nalywajko T, Austin CA, Williams MB, Ayala JE, Williams R, Lindsley SW, Conn PJ. Discovery, characterization, and antiparkinsonian effects of novel positive allosteric modulators of metabotropic glutamate receptor 4. *Molecular Pharmacology* 2008, 74(5):1345-1358. **PMCID:PMC2574552**
3. Jones CK, Bubser M, Thompson, AD, Dickerson JW, Blobaum AL, Bridges TM, Morrison RD, Daniels JS, Jadhav S, Engers DW, Italiano K, Bode J, Lindsley CW, Hopkins CR, Conn PJ, **Niswender CM**. The mGlu4 positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine A2A antagonist in preclinical rodent models of Parkinson's disease. *Journal of Pharmacology and Experimental Therapeutics* 2012, 340(2):404-421. **PMCID:PMC3263969**
4. **Patent US20130096110** Pyrazolopyridine, Pyrazolpyrazine, Pyrazolopyrimidine, Pyrazolothiophene, and Pyrazolpthiazole Compounds as mGluR4 Allosteric Potentiators, Compositions, and Methods of Treating Neurological Dysfunction. Issued 4/8/2013, most recent of 14 $mGlu_4$ patents to publish.

Identification of mGlu receptor heterodimers within the mammalian CNS

The metabotropic glutamate receptors (mGlus) are family C G protein coupled receptors (GPCRs) that function as constitutive dimers. Until recently, mGlus were thought to assemble strictly as homodimers; however, it was reported in 2011 that group II and group III receptors could heterodimerize (Doumazane et al., 2011; Kammermeier et al., 2012). These initial studies were performed in cell lines and it was unclear if heterodimerization existed in native tissues and, in particular, within the central nervous system where the majority of the mGlus are expressed. Using co-immunoprecipitation studies, we showed that $mGlu_2$ and $mGlu_4$, prototypical members of the group II and group III mGlu subfamilies, could specifically interact in rodent brain. Our additional studies in cell lines clearly showed that there are at least two classes of $mGlu_4$ PAMs, those that can potentiate both $mGlu_4$ homomers and $mGlu_{2/4}$ heteromers, and those that ONLY potentiate $mGlu_4$ homomers. In the latter case, $mGlu_4$ PAMs that shifted a glutamate concentration-response curve 40- to 50-fold to the left in efficacy experiments were without effect when $mGlu_2$ and $mGlu_4$ were co-expressed. Additionally, our *in vitro* work demonstrated that $mGlu_2$ expression exerts a dominant effect on $mGlu_4$ heterodimerization. For example, transfection of a 1:10 ratio of $mGlu_2$ to $mGlu_4$ resulted in a complete shift of pharmacology, suggesting that all, or almost all, of the $mGlu_4$ expressed under these conditions appeared to be assembled in heteromeric form. Furthermore, our *in vitro* studies showed that there was transactivation between the two protomers of an $mGlu_{2/4}$ dimer. We then used electrophysiology to show that, at a synaptic location known to express both $mGlu_2$ and $mGlu_4$ (corticostriatal synapses), the pharmacological profiles we had identified *in vitro* were recapitulated, with some PAMs completely losing potentiator activity at this synapse while other PAMs, identified to potentiate $mGlu_{2/4}$ heteromers *in vitro*, retaining activity. These experiments provide crucial evidence that $mGlu_2$ and $mGlu_4$ heterodimerize *in vivo*. These findings are paradigm-shifting for the mGlu field and suggest that the biology and pharmacology of these receptors is far more complicated than had been previously appreciated.

1. Yin S, Noetzel M, Zamorano R, Myers-Johnson KA, Conn PJ, **Niswender CM**. Selective actions of novel allosteric modulators reveal functional heteromers of metabotropic glutamate receptors in the CNS. *Journal of Neuroscience* 2014, 34(1):79-94.

Development of tools for probing the biology of metabotropic glutamate receptor 7 and identification of the receptor as a therapeutic target in Rett syndrome

I have long been interested in the biology and pharmacology of metabotropic glutamate receptors 7 and 8, receptors for which pharmacological tools have lagged behind in development compared to the other mGlus. $mGlu_7$ is highly expressed in the presynaptic active zones of both excitatory and inhibitory synapses and activation of the receptor regulates the release of both glutamate and GABA. $mGlu_7$ is thought to be a relevant therapeutic target in a number of neurological and psychiatric disorders, and polymorphisms in the *GRM7* gene have been linked to autism, depression, ADHD and schizophrenia. Via high throughput screening and chemical optimization campaigns, we have developed and released to the scientific community the first two compounds that potentiate $mGlu_7$ activity *in vitro*. While these compounds, termed VU0155094 and VU0422288, potentiate each of the group III mGlus ($mGlu_{4,6,7 \text{ and } 8}$), we have found them to be critically useful for *in vitro* and native tissue studies of $mGlu_7$ as well as the other receptors. For example, we were able to show for the first time that probe dependence (i.e., differential activity between various allosteric modulator/orthosteric agonist pairs) exists for the group III mGlus *in vitro*. By pairing studies of these nonselective compounds with a synapse in the hippocampus that expresses only $mGlu_7$, we have validated activity of these compounds in a native tissue setting.

We have since used these tools, as well as several new agonists and antagonists recently reported in the literature, to address the role of $mGlu_7$ in modulating synaptic transmission at Schaffer Collateral (SC)-CA1 terminals. $mGlu_7$ is the only mGlu expressed presynaptically at the SC-CA1 synapse in the hippocampus in adult animals and, until recently, was thought to be the predominant autoreceptor responsible for regulating glutamate release at SC terminals. We used a novel, selective $mGlu_7$ negative allosteric modulator (NAM), ADX71743, and a newly described group III agonist, LSP4-2022, to elucidate the role of $mGlu_7$ in modulating transmission in hippocampal area CA1 and have found that engagement of $mGlu_7$ is absolutely required for the induction of long term potentiation at SC-CA1 synapses. Surprisingly, this occurs mechanistically via $mGlu_7$'s ability to alter GABA, rather than glutamate, release. Taken together, these data suggest that $mGlu_7$ serves as a heteroceptor at inhibitory synapses in area CA1 and that the predominant effect of activation of $mGlu_7$ by stimulation of glutamatergic afferents is disinhibition, rather than reduced excitatory transmission. Furthermore, this $mGlu_7$-mediated disinhibition is required for induction of LTP at the SC-CA1 synapse, suggesting that $mGlu_7$ could serve as a novel therapeutic target for treatment of cognitive disorders.

We have recently found that the *GRM7* gene is positively regulated by MECP2; mutations in this protein are responsible for the majority of cases of the neurodevelopmental disorder, Rett syndrome. $mGlu_7$ protein and functional levels are greatly reduced in mice modeling the disorder. Excitingly, we have found that we can rescue hippocampal synaptic and behavioral deficits with small molecule PAMs of $mGlu_7$. This suggests that mGlu7 represents a new therapeutic direction for Rett.

1. **Niswender CM**, Johnson KA, Ayala JE, Luo Q, Williams R, Saleh S, Orton D, Weaver CD, Conn PJ. Context-dependent pharmacology induced by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol Pharmacol***,** 2010, 77(3):459-468. **PMCID: PMC2835423**
2. Jalan-Sakrikar, N, Field JP, Klar R, Mattman ME, Gregory KJ, Zamorano R, Engers DW, Bollinger SR, Weaver CD, Days EL, Lewis LM, Utley TJ, Hurtado M, Rigault D, Acher R, Walker AG, Melancon BJ, Wood MR, Lindsley CW, Conn PJ, Xiang Z, Hopkins CR, **Niswender CM**. Identification of Positive Allosteric Modulators VU0155094 (ML397) and VU0422288 (ML396) Reveals New Insights into the Biology of Metabotropic Glutamate Receptor 7. *ACS Chem Neurosci* 2014, 5(12): 1221-1237. **Free article**
3. Klar R, Walker AG, Ghose D, Grueter BA, Engers DW, Hopkins CR, Lindsley CW, Xiang, Z. Conn PJ*, **Niswender CM***. Activation of Metabotropic Glutamate Receptor 7 is required for Induction of Long Term Potentiation at SC-CA1 Synapses in the Hippocampus, *J Neurosci*, 35(19):7600-15 *****co-corresponding authors **PMCID: PMC4429158**
4. Klar R, Gogliotto R, Zamorano R, Walker AG, Blobaum AL, Engers DW, Hopkins CR, Daniels JS, Lindsley CW, Xiang Z, Conn PJ, **Niswender CM**. Identification of Metabotropic Glutamate Receptor 7 as a therapeutic target for Rett Syndrome: Rescue of Deficits in Hippocampal Long Term Potentiation and Cognition through Allosteric Modulation. *Submitted*.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/pubmed/?term=niswender+c+or+burns+cm+and+vanderbilt

D. Research Support

Ongoing

Autism Speaks (CM Niswender, PI) 03/01/2014-02/28/2017
Balancing excitotoxicity and hypoconnectivity: A case for metabotropic glutamate receptor modulation in Rett syndrome

This grant is to examine the potentiation of metabotropic glutamate receptor 5 activity in reversing the late-stage symptoms of Rett syndrome. Additionally, we hypothesize that antagonizing $mGlu_5$ in the earlier stages of the disease may prevent developmental regression. There is no overlap with the current proposal.

1R21 MH102548 (CM Niswender, PI) 08/01/2014-07/31/2016
NIMH
Metabotropic Glutamate Receptor Regulation in MeCP2-related Disorders

This application is focused on the opposing roles of $mGlu_7$ in two opposing disorders: Rett syndrome, in which the transcription factor MeCP2 is lacking, and MeCP2 Duplication syndrome. Using small molecule modulators of $mGlu_7$, we propose to test the hypothesis that potentiation of $mGlu_7$ may be beneficial in Rett, while antagonism of $mGlu_7$ may serve as a novel therapeutic strategy for MeCP2 Duplication syndrome.

Completed

1R21 NS078262-01 (CM Niswender, PI) 04/01/2012-03/31/2014
NIH/NINDS
Metabotropic Glutamate Receptors in the Basal Ganglia

Rett Syndrome (CM Niswender, PI) 01/01/2012-12/31/2013
International Rett Syndrome Foundation
Metabotropic Glutamate Receptor 7: A Potential Novel Candidate for the Treatment of Rett Syndrome

1R21 NS053536 (CM Niswender, PI) 09/20/2005-02/28/2009
NINDS
Assay for HTS of Gi/Go-linked GPCRs: mGluR7 as prototype

1X01 MH077607-01 (CM Niswender, PI) 02/01/2006-01/31/2007
NIMH
Molecular Libraries Screening Access Grant

1R03 MH076398 (CM Niswender, PI) 9/15/2005-09/14/2006
NIMH
Molecular Libraries Screening Access Grant

1F32 DK010005 (CM Niswender, PI) 03/01/2000-02/28/2003
NIDDK
Regulated expression of constitutively active PKA