BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Robert L. Macdonald, M.D., Ph.D.	Professor and Chair of Neurology Professor of Molecular Physiology and Biophysics Professor of Pharmacology
eRA COMMONS USER NAME (credential, e.g., agency login) macdonrl	

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
Mass Inst of Technology, Cambridge, MA	S.B.	1966	Electrical Engineering
Univ. of Virginia, Charlottesville, VA	Ph.D.	1969	Physiology
Univ. of Virginia, Charlottesville, VA	Postdoc Fellow	1969-1970	Neurophysiology
Univ. of Virginia, Charlottesville, VA	M.D.	1973	Medicine
Univ. of Virginia, Charlottesville, VA	Med Intern	1974	Medicine
Univ. of Virginia, Charlottesville, VA	Resident	1974-1977	Neurology

A. Personal Statement

The goal of the proposed research in this competitive renewal application is to determine the molecular pathophysiology of and to develop novel treatments for epileptic encephalopathies associated with mutations in $GABA_A$ receptor subunit genes (*GABRs*). Specifically, we propose to focus on development of the epileptic encephalopathy Dravet syndrome caused by the nonsense mutation Q390X in *GABRG2.* To understand the development of epileptic encephalopathies, we propose an *in vivo* approach using het knock-in (KI) mice expressing the mutation *Gabrg2(Q390X)* (model Dravet syndrome) using the slice electrophysiology, video EEG, western blotting and the powerful RNA-Seq technique. We will also determine if overexpression of wild type γ2 subunits can prevent development of epilepsy in the KI mice.

I have extensive experience in research on $GABA_A$ receptors and have published 236 peer reviewed papers, the majority on $GABA_A$ receptors. Over the past 10 years, I have focused much of my research on developing techniques and approaches to study the molecular pathophysiology of *GABR* mutations associated with GEs *in vitro* and with these studies to identify molecular targets for development of novel therapies for GEs. As PI on several previous and current NIH-funded grants, I have developed the regents and techniques required to study alterations in $GABA_A$ receptor function produced by these epilepsy mutations and the molecular tools to develop new approaches to therapy. I have will have completed the specific aims of the current grant and have outlined logical extensions of our *in vitro* studies to KI mice. In summary, I have a record of successful and productive epilepsy research with a focus on function and pharmacology of $GABA_A$ receptors, pathophysiology of $GABA_A$ receptor mutations associated with GEs and novel treatment of GEs.

B. Positions and Honors

Positions

1970-72 Assistant Professor of Physiology, School of Medicine, University of Virginia
1976-78 Research Assistant Professor of Neurology, School of Medicine, University of Virginia
1976-78 Research Associate, Laboratory of Developmental Neurobiology, NICHHD, NIH
1978-81 Associate Professor of Neurology, School of Medicine, University of Michigan
1981-01 Professor, Department of Neurology, University of Michigan
1982-01 Professor, Department of Physiology, University of Michigan
1995-01 Russell N. DeJong Professor of Neurology, University of Michigan
2001- Professor and Chair, Department of Neurology, Vanderbilt University
2001- Professor of Molecular Physiology and Biophysics, Vanderbilt University
2001- Professor of Pharmacology, Vanderbilt University
2014 Gerald M. Fenichel Professor of Neurology, Vanderbilt University

Other Experience and Professional Memberships

1981-83 VA Neurobiology Merit Review Board
1991-95 NIH NLS-2 Study Section
1999 NIH MDCN-5 Study Section (ad hoc)

Honors

1973 Alpha Omega Alpha
1978 S. Weir Mitchell Award, American Academy of Neurology
1986 Epilepsy Research Award, American Society of Pharmacology & Experimental Therapeutics
1993 Lennox Lecture, American Epilepsy Society
1995 Russell N. DeJong Professor of Neurology
1996 George C. Cotzias Award & Lecture, American Academy of Neurology
1997-98 President, American Epilepsy Society
1997 American Epilepsy Society Basic Research Award
1998 University of Michigan Biomedical Research Council Distinguished Faculty Award & Lecture
2000 Epilepsy of Michigan Lifetime Achievement Award
2002 Association of American Physicians
2008 Wartenberg Award & Lecture, American Academy of Neurology
2009-11 President, American Neurological Association

C. Contribution to Science (from 235 peer reviewed papers)

C.1. Characterizing the fundamental biophysical and pharmacological properties of $GABA_A$ receptors.

$GABA_A$ receptor channels mediate most inhibition in the vertebrate CNS. The receptors are heteropentamers composed of 3 subunits from a panel of 19 genes that confer different biophysical and pharmacological properties to the receptors. I have been exploring the properties of these receptors for the past 40 years. We discovered that benzodiazepines and a number of convulsant drugs act by enhancing or inhibiting $GABA_A$ receptor currents, respectively (1-4). Using single channel recording, we characterized the action of these drugs on $GABA_A$ receptor single channels (5-9, 12). We were also the first to show ordered assembly of the receptors and to characterize the properties of receptors with known subunit composition (10, 11, 13-25).

1. Macdonald RL, Barker JL: Pentylenetetrazol and penicillin are selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurons. Nature 267:720-721, 1977
2. Macdonald RL, Barker JL: Benzodiazepines specifically modulate GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Nature 271:563-564, 1978
3. Macdonald RL, Barker JL: Different action of anticonvulsant and anesthetic barbiturates revealed by use of cultured mammalian neurons. Science 200:775-777, 1978
4. Twyman RE, Rogers CJ, Macdonald RL: Differential regulation of γ-aminobutyric receptor channels by diazepam and phenobarbital. Ann Neurol 25:213-220, 1989.
5. Macdonald RL, Rogers CJ, Twyman RE: Kinetic properties of the $GABA_A$ receptor main conductance state of mouse spinal cord neurones in cell culture. J Physiol (Lond.) 410:479-499, 1989.
6. Macdonald RL, Rogers CJ, Twyman RE: Barbiturate regulation of kinetic properties of the main conductance state of $GABA_A$ receptor channel of mouse spinal neurones in culture. J Physiol (Lond.) 417:483-500, l989.
7. Twyman RE, Rogers CJ, Macdonald RL: Intraburst kinetic properties of the $GABA_A$ receptor main conductance state of mouse spinal cord neurones in culture. J Physiol (Lond) 423:193-219, l990.
8. Twyman RE, Green RM, Macdonald RL: Kinetics of open channel block by penicillin of single $GABA_A$ receptor channels from mouse spinal cord neurones in culture. J Physiol (Lond) 445:97-127, 1992.
9. Twyman RE, Macdonald RL: Neurosteroid regulation of $GABA_A$ receptor single channel kinetic properties. J Physiol (Lond) 456:215-245, 1992.
10. Angelotti TP, Uhler MD, Macdonald RL: Assembly of $GABA_A$ receptor subunits: Analysis of transient single cell expression utilizing a fluorescent substrate/marker gene combination. J Neurosci 13:1418-1428, 1993.
11. Angelotti TP, Macdonald RL: Assembly of $GABA_A$ receptor subunits: $\alpha1\beta1$ and $\alpha1\beta1\gamma2S$ subunits produce unique ion channels with dissimilar single-channel properties. J Neurosci 13:1429-1440, 1993.
12. Rogers CJ, Twyman RE, Macdonald RL: Benzodiazepine and beta-carboline regulation of single $GABA_A$ receptor channels of mouse spinal neurones in culture. J Physiol (Lond) 475:69-82, 1994.
13. Saxena NC, Macdonald RL: Assembly of $GABA_A$ receptor subunits: role of the delta subunit. J Neurosci 14:7077-7086, 1994.
14. Saxena NC, Macdonald RL: Properties of putative cerebellar γ-aminobutyric $acid_A$ receptor isoforms. Mol Pharmacol, 49:567-579, 1996.
15. Haas K, Macdonald RL: $GABA_A$ receptor subunit $\gamma2$ and δ subtypes confer unique kinetic properties on recombinant $GABA_A$ receptor currents in mouse fibroblasts. J Physiol (Lond) 514.1:27-45, 1999.

16. Bianchi MT, Haas K, Macdonald RL: Structural determinants of fast desensitization and desensitization-deactivation coupling in $GABA_A$ receptors. J Neurosci 21:1127-1136, 2001.
17. Bianchi MT, Macdonald RL: Agonist trapping by $GABA_A$ receptor channels. J Neurosci, 21:9083-9091, 2001.
18. Bianchi MT, Macdonald RL: Slow phases of $GABA_A$ receptor desensitization: Structural determinants and relevance for synaptic function. J Physiol (Lond), 544.1:3-18, 2002.
19. Lagrange AH, Botzolakis EJ, Macdonald RL: Enhanced macroscopic desensitization shapes the response of α4 subtype-containing $GABA_A$ receptors to synaptic and extrasynaptic GABA. J Physiol (Lond), 578.3:655-676, 2007. PMCID: PMC2151343
20. Bianchi MT, Botzolakis EJ, Haas K, Fisher JL, Macdonald RL: Microscopic kinetic determinants of macroscopic currents: Insights from coupling and uncoupling of $GABA_A$ receptor desensitization and deactivation. J Physiol (Lond) 584.3: 769–787, 2007. PMCID: PMC2276985
21. Botzolakis, EJ, Maheshwari A, Feng HJ, Lagrange A, Shaver J, Kassebaum N, Baudenbacher F, Macdonald RL: Achieving synaptically-relevant pulses of neurotransmitter using PDMS microfluidics. J Neurosci Methods, 177:294-302, 2009. PMID: 19013195
22. Bianchi MT, Botzolakis, EJ Lagrange AH, and Macdonald RL: The effect of benzodiazepines on $GABA_A$ receptor opening frequency: Differential modulation under synaptic and extrasynaptic conditions. Epilepsy Res, 85: 212-220, 2009. PMCID: 2834588. PMCID: PMC2834588
23. Lo WY, Lagrange AH, Hernandez CC, Harrison R, Dell A, Haslam SM, Sheehan JH, Macdonald RL: Glycosylation of β2 subunits regulates $GABA_A$ receptor biogenesis and channel gating. J Biol Chem 285:31348-31361,2010. PMCID: PMC2951209
24. Wu X, Ning G, Guo Y, Ali R, Macdonald RL, De Blas AL, Luscher B, Chen G: Molecular mechanisms governing synaptic versus extrasynaptic targeting of $GABA_A$ receptors. J Biol Chem, 287:27417-30, 2012.
25. Lo W-Y, Lagrange AH, Hernandez CC, Gurba KN, and Macdonald RL: Coexpression of γ2 subunits prevents processing of N-linked glycans attached to the N104 glycosylation sites of $GABA_A$ receptor β2 subunits. Neuro Chem Res, 39:1088-103, 2014.

C.2. Characterization of the mechanisms of GABR mutations in genetic epilepsies.

Monogenic familial genetic epilepsies were shown to be associated with mutations in GABAA receptor genes (GABRs) in 2001 and we have been unraveling the pathogenic mechanisms of these mutations. We have demonstrated the fundamental pathogenesis of both missense and nonsense mutations and placed the mechanisms into 6 classes: 1) impaired transcription , 2) impaired translation (11, 21, 24, 26), 3) Impaired folding and assembly (3, 5, 9, 11, 17), 4) truncation with unstable protein or with stable truncated protein and dominant negative effects (12, 13, 16, 23, 25), 5) defective assembly/trafficking (1, 2, 4, 8, 10, 18-20, 27, 28) and 6) impaired channel gating (1, 6, 19, 20)

1. Bianchi MT, Song L, Zhang H, Macdonald RL: Two different mechanisms of disinhibition produced by $GABA_A$ receptor mutations linked to epilepsy in humans. J Neurosci, 22:5321-5327, 2002.
2. Dibbens LM, Hua-Jun Feng H-J, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC: GABRD encoding a protein for extra-synaptic $GABA_A$ receptors is a susceptibility locus for Generalized Epilepsies. Human Molecular Genetics, 13:1315-1319 2004.
3. Gallagher MJ, Song L, Macdonald RL: The juvenile myoclonic epilepsy $GABA_A$ receptor α1 subunit mutation A322D produces asymmetrical, subunit position-dependent reduction of heterozygous receptor currents and α1 subunit protein. J Neurosci., 24:5570-5578, 2004.
4. Kang J, Macdonald RL: The $GABA_A$ receptor γ2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures causes retention of $\alpha1\beta2\gamma2$ receptors in the endoplasmic reticulum. J Neurosci, 24:8672-8677, 2004.
5. Gallagher MJ, Song L, Shen W, Macdonald RL: Endoplasmic reticulum retention and associated degradation of a $GABA_A$ receptor epilepsy mutation that inserts an aspartate in the M3 transmembrane segment of the α1 subunit. J Biol Chem, 280: 37995-38004, 2005.
6. Feng H-J, Kang J-Q, Song L, Dibbens L, Mulley J, Macdonald RL: The δ subunit susceptibility variants E177A and R220H associated with complex epilepsy alter channel gating and cause endoplasmic reticulum retention of $\alpha4\beta2\delta$ $GABA_A$ receptors. J Neurosci, 26: 1499-1506, 2006.
7. Kang J-Q, Shen W, Macdonald RL: Why does fever trigger seizures?: $GABA_A$ receptor γ2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. J Neurosci, 26:2590-2597, 2006.

8. Audenaert D, Schwartz E, Claeys KG, Claes L, Deprez L, Suls A, Van Dyck T, Lagae L, Van Broeckhoven C, Macdonald RL, De Jonghe P: The GABRG2 gene and febrile seizures: extension of the mutational spectrum. Neurology, 67:687-90, 2006.
9. Gallagher MJ, Ding L, Maheshwari A, Macdonald RL: The $GABA_A$ Receptor α1 subunit epilepsy mutation A322D inhibits transmembrane helix formation and causes proteasomal degradation. Proc Natl Acad Sci, 104:12999-13004, 2007. PMCID: PMC1941799\
10. Tanaka M, Olsen RW, Medina MT, Schwartz E, Alonso ME, Duron RM, Castro-Ortega R, Martinez-Juarez IE, Pascual-Castroviejo E, Machado-Salas J, Silva R, Bailey JN, Bai D, Ochoa A, Jara-Prado A, Pineda G, Macdonald RL and Delgado-Escueta AV: Hyperglycosylation and reduced GABA currents of mutated GABRB3 polypeptide in remitting childhood absence epilepsy. Amer J of Human Genetics 82:1249-1261, 2008. PMCID: 2668111
11. Kang J-Q, Shen W, Macdonald RL: Two molecular pathways (NMD and ERAD) contribute to a genetic epilepsy associated with the $GABA_A$ receptor GABRA1 PTC mutation, 975delC, S326fs328X. J Neurosci, 29: 2833-2844, 2009. PMCID: PMC2687144
12. Kang J-Q, Shen W, Macdonald RL: The GABRG2 mutation, Q351X, associated with GEFS+ has both loss of function and dominant-negative suppression. J Neurosci, 29: 2845-2856, 2009. PMCID: 2754234
13. Kang J-Q, Macdonald RL: Molecular pathology of GABR genes with premature translation-termination codon (PTC) mutations associated with genetic epilepsies. Trends in Molec Medicine, 15.9:430-438, 2009. PMCID: PMC3076198.
14. Belelli D, Harrison N, Maguire J, Macdonald RL, Walker M, Cope D: Extrasynaptic $GABA_A$ receptors: Form, Pharmacology and Function. J Neurosci, 29: 12757-12763, 2009. PMCID: PMC2784229
15. Macdonald RL, Kang J-Q, Gallagher MJ: Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. J Physiol (Lond), 588:1861-1869, 2010. PMCID: PMC2901974
16. Kang J-Q, Shen W, Lee M, Gallagher M, Macdonald RL: Slow degradation and aggregation of mutant $GABA_A$ receptor γ2(Q351X) subunits associated with epilepsy: additional mechanisms of neuronal disinhibition? J Neurosci, 30:13895-13905, 2010. PMCID: 2976503
17. Ding, L, Feng, HJ, Macdonald, RL, Botzolakis, EJ, Hu, N, Gallagher, MJ: GABA(A) receptor alpha1 subunit mutation A322D associated with autosomal dominant juvenile myoclonic epilepsy reduces the expression and alters the composition of wild type GABA(A) receptors. J Biol Chem, 285:26390-26405, 2010. PMCID: PMC2924069
18. Delahanty RJ, Kang J-Q, Brune CW, Kistner EO, Courchesne E, Macdonald RL, Sutcliffe JS: Maternal transmission of a rare GABRB3 signal peptide variant is associated with autism. Molecular Psychiatry, 16:86-96, 2011. PMCID:2822691
19. Hernandez CC, Gurba KN, Hu N, Macdonald RL: The GABRA6 mutation, R46W, associated with childhood absence epilepsy alters $\alpha6\beta2\gamma2$ and $\alpha6\beta2\delta$ $GABA_A$ receptor channel gating and assembly. J Physiol (Lond), 589:5857-5878, 2011. PMCID: PMC3249055
20. Gurba KN, Hernandez CC, Hu N, Macdonald RL: The GABRB3 mutation, G32R, associated with childhood absence epilepsy, alters α1β3γ2L $GABA_A$ receptor channel gating and expression. J Biol Chem, 287: 12083-12097, 2012. PMCID: PMC3320954
21. Tian M, Macdonald RL: The intronic GABRG2 mutation, IVS6+2T→G, associated with CAE altered $\gamma2$ subunit mRNA intron splicing and activated nonsense-mediated mRNA decay and altered splicing. J Neurosci, 32:5937-5952, 2012. PMCID: PMC3357398
22. Macdonald RL, Kang J-Q: mRNA Surveillance and ER quality control processes alter biogenesis of mutant $GABA_A$ receptor subunits associated with genetic epilepsies. Epilepsia, 53 Suppl 9:59-70, 2012 PMID 23216579.
23. Huang X*, Tian M*, Hernandez C, Hu N and Macdonald RL: The Dravet syndrome-associated *GABRG2* nonsense mutation, Q40X, activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through. Neurobiology of Disease, 48:115-123, 2012.
24. *Tian M, *Mei D, Freri E, Hernandez CC, Granata T, Shen W, †Macdonald RL, †Guerrini R: Impaired surface αβγ GABA(A) receptor expression in familial epilepsy due to a GABRG2 frameshift mutation. Neurobiology of Disease, 50:135-141, 2012.
25. Kang J-Q, Shen W, Macdonald RL: GABRG2 nonsense epilepsy mutations, W429X and Q390X, produce trafficking-deficient $\gamma2$ subunits with different stabilities and gain of cellular toxicity: implications for phenotypic variation. Ann Neurol, 74:547-59, 2013. PMID 23720301

26. *Johnston JA, *Kang J-Q, Shen W, Pickrell WO, Cushion TD, Davies JS, Baer K, Mullins JGL, Hammond CL, Chung SK, Thomas RH, White C, Smith PEM, †Macdonald RL, †Rees MI: A Novel GABRG2 Mutation, p.R136*, in a family with GEFS+ and extended phenotypes. Neurobiology of Disease, 64:131–141, 2014.
27. Huang X, Hernandez CC, Hu N, Macdonald RL: Three epilepsy-associated *GABRG2* missense mutations at the γ+/β- interface disrupt $GABA_A$ receptor assembly and trafficking by similar mechanisms but to different extents. Neurobiology of Disease, 68:167-179, 2014.
28. Todd E, Gurba KN, Botzolakis E, Stanic Kostic A, Macdonald RL: $GABA_A$ receptor biogenesis is impaired by the γ2 subunit febrile seizure-associated mutation, *GABRG2(R177G)*. Neurobiology of Disease, 69:215-24, 2014.
29. Kang J-Q, Shen W, Zhou C, Xu D, Macdonald RL: The human epilepsy mutation *GABRG2(Q390X)* causes chronic neurodegeneration. Nature Neuroscience, in press.

C.3. Defining the mechanisms of action of antiepileptic drugs.

We have made significant contributions to the understanding of the approach to determining clinically relevant mechanisms of action of antiepileptic drugs and to development of a mechanistic scheme of AED actions.

1. Macdonald RL, Barker JL: Enhancement of GABA-mediated postsynaptic inhibition in cultured mammalian spinal cord neurons: A common mode of anticonvulsant action. Brain Res 167:323-336, 1979.
2. Macdonald RL, Barker JL: Anticonvulsant and anesthetic barbiturates: Different postsynaptic actions in cultured mammalian neurons. Neurology 29:432-447, 1979.
3. Macdonald RL, Bergey GK: Valproic acid augments GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Brain Res 170:558-562, 1979.
4. Schulz DW, Macdonald RL: Barbiturate enhancement of GABA-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions. Brain Res 209:177-188, 1981.
5. McLean MJ, Macdonald RL: Multiple actions of phenytoin on mouse spinal cord neurons in cell culture. J Pharmacol Exp Ther 227:779-789, 1983.
6. McLean MJ, Macdonald RL: Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther 237:1001-1011, 1986.
7. McLean MJ, Macdonald, RL: Carbamazepine and 10,11-epoxycarbamazepine produce use- and voltage-dependent limitation of rapidly firing action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther 238:727-732, 1986.
8. McLean MJ, Macdonald RL: Benzodiazepines, but not beta carbolines, limit high frequency repetitive firing of action potentials of spinal cord neurons in cell culture. J Pharmacol Exp Ther 244:789-795, 1988.
9. Wohlfarth K, Bianchi MT, and Macdonald RL: Enhanced neurosteroid potentiation of ternary $GABA_A$ receptors containing the delta subunit. J Neurosci, 22:1541-1549, 2002.
10. Kapur J, Macdonald RL: Rapid seizure-induced reduction of benzodiazepine and Zn^{2+} sensitivity of hippocampal dentate granule cell $GABA_A$ receptors. J Neurosci 17:7532-7540, 1997.

D. Research Support

Ongoing Research Support

R01 NS33300-18 Macdonald (PI) 05/01/1995 – 11/30/2016
GABA(A) Receptor Assembly/Trafficking/Function and Epilepsy Missense Mutations
The goal of this study is to investigate the effects of human epilepsy mutations γ2(R82Q), γ2(P83S), γ2(N79S), and β3(P11S) on function and trafficking of αβγ2 δ $GABA_A$ receptors.

R01 NS50590-04 Macdonald (PI) 12/01/2010 – 11/30/2015
Function/trafficking of α1β2γ2 $GABA_A$Rs with γ2 subunit truncations
The goal of this study is to characterize the effects of human AD epilepsy mutations in the $GABA_A$ receptor γ2 gene (GABRG2) that introduce premature translation-termination codons (PTCs) or alter splice donor sites on the function, surface expression, and trafficking of heterozygous mutant α1β2γ2 receptors, including γ2(Q40X), γ2(Q390X) and γ2(IVS6+2T-G) mutations.

VUMC44108-R Macdonald (coPI) 09/26/2014 - 06/30/2017
Mapping neuronal chloride microdomains
The goal of this study is to characterize chloride microdomains in neurons using the chloride sensitive fluorophore Super Chlomeleon fused to $GABA_A$ receptor gamma2 and delta subunits to sense sub- and extrasynaptic chloride microdomains.