Curriculum Vitae

JOHN O'BRIEN, PH.D.

PROFESSOR AND FREDERIC B. ASCHE CHAIR IN OPHTHALMOLOGY

August 2014

WORK ADDRESS: The Richard S. Ruiz, M.D. Department of Ophthalmology
and Visual Science
University of Texas Medical School at Houston
6431 Fannin, MSB 7.024
Houston, TX 77030

CITIZENSHIP: USA

UNDERGRADUATE EDUCATION:

1985 B.A. *Summa Cum Laude*, *Summa Cum Laude* in Biochemistry, Bowdoin College, Brunswick, Maine. Thesis advisor: John L. Howland, Ph.D.

GRADUATE EDUCATION:

1991 Ph.D. in Biology, University of California, San Diego. Advisor: George N. Somero, Ph.D. (Member; National Academy of Sciences), Co-advisor: Russell D. Vetter, Ph.D.

POSTGRADUATE TRAINING:

1991-1994 University of Chicago. Advisor: Barbara A. Block, Ph.D.

1994-1998 University of Illinois, Chicago. Advisor: Harris Ripps, Ph.D., D.Sc., Co-advisor: Muayyad R. Al-Ubaidi, Ph.D.

ACADEMIC APPOINTMENTS:

1998 - 2005	Assistant Professor, Department of Ophthalmology and Visual Science, University of Texas Medical School at Houston
1998 - present	Cross-appointment, Department of Biochemistry and Molecular Biology, University of Texas Medical School at Houston
1999 - 2003	Adjunct Assistant Professor, University of Houston College of Optometry
1999 - present	Graduate Faculty, The Graduate School of Biomedical Sciences, University of Texas Health Science Center at Houston
2005 – 2011	Associate Professor with tenure, Department of Ophthalmology and Visual Science, University of Texas Medical School at Houston
2011 – present	Professor with tenure, Department of Ophthalmology and Visual Science, University of Texas Medical School at Houston

PROFESSIONAL ORGANIZATIONS:

American Association for the Advancement of Science
Association for Research in Vision and Ophthalmology

American Society for Cell Biology
Biophysical Society
Society for Neuroscience

HONORS AND AWARDS:

1982-1985	James Bowdoin Scholar (each year)
1984-1985	Surdna Foundation Student Research Fellowship
1985	Graduated *Summa cum laude; Summa cum laude* in Biochemistry
1985	Elected to Phi Beta Kappa
1985-87, 1989-91	NIH Predoctoral Fellowship (Departmental training grant)
1989	Second prize; American Society of Zoologists annual meeting best student paper award
1990	Society of Sigma Xi Grant in Aid of Research
1993-1996	NIH Postdoctoral Fellowship (Individual NRSA)
1995	1995 International Gap Junction Conference travel award
1995-1996	Illinois Eye Fund Grant in Aid of Research (Co-PI)
2005-2014	Dean's Teaching Excellence Award (UT Medical School, Houston) (7 times)
2009-present	Frederic B. Asche Chair in Ophthalmology
2010	Student Marshall for UT Houston GSBS Commencement

SERVICE ON NATIONAL/INTERNATIONL GRANT REVIEW PANELS, STUDY SECTIONS, ETC.

Ad hoc reviewer – Netherlands Organization for Health Research and Development
Ad hoc reviewer – Neurotransporters, Receptors and Calcium Signaling study section (NIH)
Canada Research Chairs Program – College of Reviewers
Ad hoc reviewer – Natural Sciences and Engineering Council of Canada
Ad hoc reviewer – United States-Israel Binational Science Foundation

SERVICE ON THE UNIVERSITY OF TEXAS MEDICAL SCHOOL, HOUSTON COMMITTEES:

Mar. 2001 – June 2001	Member - UTHMS Energy task force
Sept. 2001 – Mar. 2002	Member – Ophthalmology Dept. Faculty Search Committee
Sept. 2003 – Aug. 2006	Faculty Senate – Representative from Ophthalmology Dept.
May 2005 – Nov. 2008	Member – Ophthalmology Dept. Faculty Search Committee
Sept. 2006 – Aug 2009	Faculty Senate – At-large Associate Professor
Sept. 2009 – Aug 2012	Faculty Senate – Representative from Ophthalmology Dept.

SERVICE ON THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER, HOUSTON COMMITTEES:

March 2003 – present	Member – Biological Safety Committee (Vice-chairman since 2006)
Aug. 2008 – Apr. 2009	Member – ad hoc committee to investigate allegations of research misconduct

SERVICE ON THE UNIVERSITY OF TEXAS SYSTEM COMMITTEES:

Feb. 2004 – June 2014	Member – UT Institutional Biosafety Committee; UTHSC Houston Subcommittee (Vice-chairman since 2006)

SERVICE ON GRADUATE SCHOOL COMMITTEES:

GSBS Admissions Committee (reviews approximately 700 applicants per year in all disciplines) – member 9/2005 – 9/2008; Chairman for 2007-2008 season.

GSBS Executive Committee – member 9/2007 – 8/2009, member 9/2013 – present

GSBS Curriculum Committee – member 9/2011 – 8/2013

Neuroscience Program admissions pre-review committee – member 9/2008 – present

Neuroscience Program standing candidacy exam committee – member 1/2013 - present

GRADUATE SCHOOL AFFILIATIONS:

Program in Neuroscience (Co-director of Visual Neuroscience Track)
Program in Cell and Regulatory Biology

GRADUATE STUDENT COMMITTEES:

Advisory/Supervisory Committees – 23 committees; Chairman of 6.
Examining Committees – 19 committees; Chairman of 1.

External Thesis Examiner

Sept. 2009 – Feb. 2010	Ph.D. examining committee for Carmen Flores (Albert Einstein College of Medicine; Ph.D. 2010)
Oct. 2012 – Dec. 2012	Ph.D. Examining committee for Serra Akinturk (Ruhr Universität Bochum, Germany; Ph.D. 2012)
July 2013 – Dec. 2013	Ph.D. external examiner for Cindy Guo (University of Auckland, New Zealand; Ph.D. 2013)

TEACHING ACTIVITIES:

Lecturer: Current topics in Neuroscience (GS14 0611) – Fall 1999-2002, Fall 2004, Fall 2006, Fall 2007, Fall 2010
Facilitator: Problem Based Learning (Medical School) – Fall 1999, Fall 2001-2003, Spring 2005-2007, Fall 2007, Spring 2008-2009 (sub), Fall 2009-2010, Fall 2012-2014
Lecturer: Visual Neuroscience I (GS14 0073) – Spring 1999, Spring 2004, Spring 2005, Spring 2007, Spring 2011
Lecturer: Cellular Neurophysiology (GS14 0143) – Fall 2009-2014

TUTORIAL STUDENTS SUPERVISED:

9 GSBS graduate students, 1 University of Houston graduate student, 4 summer undergraduate research students, 3 UTH Medical School summer research students, 2 external summer medical student researchers, 1 international (Oldenburg University – Germany) post-baccalaureate research internship student, 1 summer high school research student

SPONSORSHIP OF CANDIDATES FOR POSTGRADUATE DEGREE:

Virginia Winbow (U. of Houston) – MS in Optometry 2002
William W. Kothmann (GSBS) – Ph.D. 2010
Yanran (Helen) Wang (GSBS) – Ph.D. candidate
Alejandro Vila (GSBS) – Ph.D. candidate
Joshua Atkinson (UTH Medical School) – Scholarly focus in Neuroscience; MD expected in 2015.

SPONSORSHIP OF POSTDOCTORAL FELLOWS:

Mohammed S. Choudhary, M.D., Ph.D.	2002
Xiaosen Ouyang, M.D.	2003
C. Steven Miller, Ph.D.	2006-2009
Xiaofan Li, Ph.D.	2006-2009
Hongyan Li, Ph.D.	2007-2013
Shunichi Yoshikawa, Ph.D	2013-present
Keith Moore, Ph.D.	2013-present

OTHER MEDICAL SCHOOL SERVICE:

Interview Medical School Candidates: 2000/2001 – 2005/2006 seasons

OTHER SCIENTIFIC SERVICE:

Referee for the following journals:
BMC Developmental Biology
Biochimica et Biophysica Acta
Biological Bulletin
Biophysical Journal
Brain Research
Cell Communication and Adhesion
Experimental Eye Research
Frontiers in Cellular Neuroscience
Investigative Ophthalmology & Visual Science
Journal of Biological Chemistry
Journal of Calcium Binding Proteins
Journal of Cell Science
Journal of Comparative Neurology
Journal of Experimental Biology
Journal of Neurochemistry
Journal of Neurophysiology

Journal of Neuroscience
Journal of Neuroscience Methods
Journal of Neuroscience Research
Journal of Physiology
Molecular Vision
Neuro-Signals
PLOS One
Proceedings of the National Academy of Sciences
Trends in Neuroscience
Vision Research
Visual Neuroscience

CURRENT GRANT SUPPORT:

Active:

NIH, National Eye Institute 2R01 EY012857-12
P.I.: John O'Brien
Title: "Regulation of retinal gap junctions"
Award period: 4/1/10 – 3/31/14; no-cost extension to 3/31/15

The goals of this project are to understand the molecular mechanisms that regulate electrical coupling in retinal neurons. This proposal focuses on the signaling mechanisms that control gap junction coupling in retinal photoreceptors and amacrine cells during light adaptation. Signaling mechanisms to be examined include protein kinase signaling pathways and the connexin-associated protein complexes that may control these signaling pathways.

NIH, National Eye Institute 2P30 EY010608-18
Title: "Core Grant for Vision Research"
P.I.: Stephen C. Massey
Award period: 03/01/09 – 02/28/14
John O'Brien role: Director, Tissue Culture Module

This award supports vision research by a consortium of investigators through shared use facilities for cell and tissue culture, microarray studies, confocal microscopy, electron microscopy, computing, and biostatistics.

NIH, National Institute for Neurological Diseases and Stroke 1R21 NS085772-01
P.I.: Alberto Pereda
Subcontract P.I.: John O'Brien
Title: "Generation of transgenic zebrafish to study electrical synaptic transmission"
Award period: 12/1/13 – 11/30/15

The goals of this project are to generate transgenic zebrafish in which connexins involved in Mauthner cell electrical synapses are tagged with fluorescent proteins and to study the role of connexin turnover in plasticity of electrical synapses. My role in this project is to generate the transgenic zebrafish to be used for the experiments.

Pending:

NIH, National Eye Institute 5 R01 EY012857-13
P.I.: John O'Brien
Title: "Regulation of retinal gap junctions"
Award period: 4/1/14 – 3/31/19

The goals of this project are to understand the molecular mechanisms that regulate electrical coupling in retinal neurons. This proposal focuses on the signaling mechanisms that control gap junction coupling in retinal photoreceptors and amacrine cells during light adaptation. Signaling mechanisms to be examined include protein kinase signaling pathways and the connexin-associated protein complexes that may control these signaling pathways.

PAST GRANT SUPPORT:

NIH, National Institute of Neurological Diseases and Stroke 1 F31 NS063534-01
P.I.: William W. Kothmann
Sponsor: John O'Brien
Award Period: 9/1/08 – 8/31/10
Title: "Regulation of connexin35/36-mediated coupling by phosphorylation"

This was an individual predoctoral fellowship that provided a stipend and some research support.

American Health Assistance Foundation, Macular Degeneration Research Program
P.I.: John O'Brien M2006-25
Title: "Physiological uncoupling of cone gap junctions"
Award period: 4/1/06 – 3/31/08 (no-cost extension to 3/31/09)

This project was directed towards characterizing the role of cone photoreceptor coupling in the spread of photoreceptor degeneration. Experiments determined physiological conditions and pharmacological treatments control cone photoreceptor gap junction coupling. Further experiments established a light exposure model of photoreceptor degeneration to study the role gap junctions in retinal degeneration.

NIH, National Center for Research Resources 1 G20 RR024000-01
P.I.: John O'Brien
Title: "Facilities for research use of fish"
Award period 07/15/07 – 07/14/08

This award established a fully equipped transgenic zebrafish facility within the Animal Care Center at the University of Texas Health Science Center at Houston. This is the first institutional zebrafish facility in any of the Texas Medical Center institutions. Components include conventional housing, circulation, and water treatment systems, breeding equipment, and DNA microinjection equipment. Additional facilities for other freshwater fish species were also included.

Career Development Award; Research to Prevent Blindness
P.I.: John O'Brien
Title: "In vivo analysis of connexin expression in the rabbit retina"

Award period: 1/1/99 – 12/31/02

NIH, NIAMS F32 AR08254
P.I.: John O'Brien Sponsor: Harris Ripps
Title: "Molecular Characterization of Gap Junction Proteins"
Award Period: 11/20/94 – 11/19/96

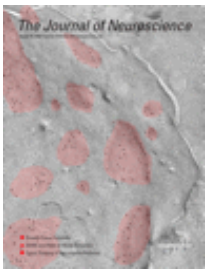
NIH, NIAMS F32 AR08254
P.I.: John O'Brien Sponsor: Barbara A. Block
Title: "Functional Roles of Ryanodine Receptor Isoforms"
Award Period: 11/20/93 – 11/19/94

PUBLICATIONS:

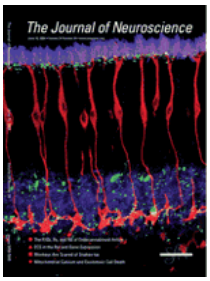
Original Research Articles

1. **O'Brien, J.**, and Vetter, R.D.: Production of thiosulphate during sulphide oxidation by mitochondria of the symbiont-containing bivalve *Solemya reidi*. J. Exp. Biol. *149:* 133-148, 1990.
2. **O'Brien, J.**, Dahlhoff, E., and Somero, G.N.: Thermal resistance of mitochondrial respiration: hydrophobic interactions of membrane proteins may limit thermal tolerance. Physiol. Zool. *64(6):* 1509-1526, 1991.
3. Dahlhoff, E., **O'Brien, J.**, Somero, G.N., and Vetter, R.D.: Temperature effects on mitochondria from hydrothermal vent invertebrates: evidence for adaptation to elevated and variable habitat temperatures. Physiol. Zool. *64(6):* 1490-1508, 1991.
4. **O'Brien, J.**, Meissner, G., and Block, B.A.: The fastest contracting skeletal muscles of non-mammalian vertebrates express only one isoform of the ryanodine receptor. Biophys. J. 65(12): 2418-2427, 1993. PMCID: PMC1225982
5. Block, B.A., **O'Brien, J.**, and Meissner, G.: Characterization of the sarcoplasmic reticulum proteins in the thermogenic muscles of fish. J. Cell Biol. *127(5):* 1275-1288, 1994.
6. **O'Brien, J.**, Valdivia, H.H., and Block, B.A.: Physiological differences between the α and β ryanodine receptors of fish skeletal muscle. Biophys. J. 68(2): 471-482, 1995. PMCID: PMC1281711
7. **O'Brien, J.**, Al-Ubaidi, M.R., and Ripps, H.: Connexin 35: A gap junctional protein expressed preferentially in the skate retina. Mol. Biol. Cell 7: 233-243, 1996. PMCID: PMC275876
8. **O'Brien, J.**, and Block, B.A.: Effects of calcium on oxidative phosphorylation in mitochondria from the thermogenic organ of billfishes. J. Exp. Biol. *199(12):* 2679-2687, 1996.
9. **O'Brien, J.**, Ripps, H., and Al-Ubaidi, M.R.: Molecular cloning of a rod opsin cDNA from the skate retina. Gene 193: 141-150, 1997.
10. Qian, X., Malchow, R.P., **O'Brien, J.**, and Al-Ubaidi, M.R.: Isolation and characterization of a skate retinal GABA transporter cDNA. Mol. Vis. *4:* 6, 1998.
11. **O'Brien, J.**, Bruzzone, R., White, T.W., Al-Ubaidi, M.R., and Ripps, H.: Cloning and expression of two related connexins from the perch retina define a new subgroup of the connexin family. J. Neurosci. *18 (19):* 7625-7637, 1998.

12. White, T.W., Deans, M.R., **O'Brien, J.**, Al-Ubaidi, M.R., Goodenough, D.A., Ripps, H., and Bruzzone, R.: Functional characteristics of skate connexin35, a member of the γ subfamily of connexins expressed in the vertebrate retina. Eur. J. Neurosci. *11(6):* 1883-1890, 1999.
13. Mills, S.L., O'Brien, J.J., Li, W., **O'Brien, J.**, and Massey, S.C.: Rod pathways in the mammalian retina utilize connexin 36. J. Comp. Neurol. 436: 336-350, 2001. PMCID: PMC1922340
14. Li, C., Ding, X.-Q., **O'Brien, J.**, Al-Ubaidi, M.R. and Naash, M.I. Molecular characterization of the skate peripherin/*rds* gene: relationship to its orthologues and paralogues. Invest. Ophth. Vis. Sci. *44(6)*: 2433-2441, 2003.



15. Pereda, A., **O'Brien, J.**, Nagy, J.I., Bukauskas, F., Davidson, K.G.V., Yasumura, T., and Rash, J.E. Connexin35 mediates electrical transmission at mixed synapses on Mauthner cells. J. Neurosci. 23(20): 7489-7503, 2003. PMCID: PMC1805790
16. Naash, M.I., Ding, X.-Q., Li, C., **O'Brien, J.**, and Al-Ubaidi, M.R. Peripherin/rds in the skate retina. Adv. Exp. Med. Biol. 533:377-383, 2003.
17. **O'Brien, J.**, Nguyen, H.B., and Mills, S.L. Cone photoreceptors in bass retina use two connexins to mediate electrical coupling. J. Neurosci. 24(24): 5632-5642, 2004. PMCID: PMC2222551



18. Ouyang, X., Winbow, V.W., Patel, L.S., Burr, G.S., Mitchell, C.K., and **O'Brien, J.** Protein kinase A mediates reversible regulation of electrical synapses containing connexin35 through a complex pathway. Mol. Brain Res. 135(1-2): 1-11, 2005. PMCID: PMC2212611
19. Burr, G.S., Mitchell, C.K., Keflemariam, Y.J., Heidelberger, R., and **O'Brien, J.** Calcium-dependent binding of calmodulin to retinal gap junction proteins. Biochem. Biophys. Res. Comm. 335: 1191-1198, 2005. PMCID: PMC2222552
20. Patel, L.S., Mitchell, C.K., Dubinsky, W.P., and **O'Brien, J.** Regulation of gap junction coupling through the neuronal connexin Cx35 by nitric oxide and cGMP. Cell Commun. Adhes. 13(1-2): 41-54, 2006. PMCID: PMC2189984
21. Hoshi, H., **O'Brien, J**, and Mills, S.L. A novel fluorescent tracer for visualizing coupled cells in neural circuits of living tissue. J. Histochem. Cytochem. 54(10): 1169-1176, 2006. PMCID: PMC1851887
22. O'Brien J.J., Li W., Pan, F., Keung, J., **O'Brien J.**, and Massey S.C. Coupling between A-type horizontal cells is mediated by connexin 50 gap junctions in the rabbit retina. J. Neurosci. *26(45):* 11624-11636, 2006.
23. Kothmann, W.W.**,** X. Li, Burr, G.S., and **O'Brien, J.** Connexin35/36 is phosphorylated at regulatory sites in the retina. Vis. Neurosci. 24(3): 363-375, 2007. PMCID: PMC2170900
24. Cachope, R., Mackie, K., Triller, A., **O'Brien, J.**, and Pereda, A.E. Potentiation of electrical and chemical synaptic transmission mediated by endocannabinoids. Neuron 56: 1034-1047, 2007. PMCID: PMC2770950
25. González-Nieto, D., Gómez-Hernández, J.M., Larrosa, B., Gutiérrez, C., Muñoz, M.D., Fasciani, I., **O'Brien, J.**, Zappalà, A., Cicirata, F., and Barrio, L.C. Regulation of neuronal connexin-36 channels by pH. Proc. Nat. Acad. Sci. USA 105: 17169-17174, 2008. PMCID: PMC2579396

26. Kothmann, W.W., Massey, S.C., and **O'Brien, J.** Dopamine-stimulated dephosphorylation of connexin 36 mediates AII amacrine cell uncoupling. J. Neurosci. 29(47): 14903-14911, 2009. PMCID: PMC2839935
27. Li, H., Chuang, A.Z., and **O'Brien, J**. Photoreceptor coupling is controlled by connexin 35 phosphorylation in zebrafish retina. J. Neurosci. 29(48): 15178-15186, 2009. PMCID: PMC2909833
28. Vila, A., Satoh, H., Rangel, C., Mills, S.L., Hoshi, H., **O'Brien, J.**, Marshak, D.R., MacLeish, P.R., and Marshak, D.W. Histamine receptors of cones and horizontal cells in old world monkey retinas. J. Comp. Neurol. 520(3): 528-543, 2012. PMCID: PMC3272842
29. Pan, F., Keung, J., Kim, I.-B., Snuggs, M.B., Mills, S.L., **O'Brien, J**., and Massey, S.C. Connexin 57 is expressed by the axon terminal network of B-type horizontal cells in the rabbit retina. J. Comp. Neurol. 520(10): 2256-2274, 2012.
30. O'Brien J.J., Chen, X., MacLeish, P.R., **O'Brien J.**, and Massey S.C. Photoreceptor Coupling mediated by Connexin 36 in the Primate Retina. J. Neurosci. 32(13): 4675-4687, 2012. PMCID: PMC3335500

31. Kothmann, W.W., Trexler, E.B., Whitaker, C.M., Li, W., Massey, S.C., and **O'Brien, J.** Nonsynaptic NMDA receptors mediate activity-dependent plasticity of gap junctional coupling in the AII amacrine cell network. . J. Neurosci. 32(20): 6747-6759, 2012. PMCID: PMC3367513
32. Li, H., Zhang, Z., Blackburn, M.R., Wang, S.W., Ribelayga, C.P. and **O'Brien, J.** Adenosine and dopamine receptors co-regulate photoreceptor coupling via gap junction phosphorylation in mouse retina. J. Neurosci. 33(7): 3135-3150, 2013.
33. Palacios-Prado, N., Hoge, G, Marandykina, A., Rimkute, L., Chapuis, S., Paulauskas, N., Skeberdis, V.A., **O'Brien, J**., Pereda, A.E., Bennett, M.V.L., and Bukauskas, F.F. Intracellular magnesium-dependent modulation of gap junction channels formed by neuronal connexin36. J. Neurosci. 33(11): 4741-4753, 2013.
34. Rash, J.E., Curti, S., Davidson, K.G.V., Kamasawa, N., Nannapaneni, S., Flores, C.E., Yasumura, T., **O'Brien, J.**, Lynn, B.D., Nagy, J.I., and Pereda, A.E. Molecular and functional asymmetry at a vertebrate electrical synapse. Neuron 79(5): 957-969, 2013.
35. Li, H., Chuang, A.Z., and **O'Brien, J**. Regulation of photoreceptor gap junction phosphorylation by adenosine in zebrafish retina. Vis. Neurosci. 31(3): 237-243. doi:10.1017/S095252381300062X

Invited Articles

1. Desbruyères, D., Chevaldonné, P., Alayse, A.-M., Jollivet, D., Lallier, F.H., Jouin-Toulmond, C., Zal, F., Sarradin, P.-M., Cosson, R., Caprais, J.-C., Arndt, C., **O'Brien, J.**, Guezennec, J., Hourdez, S., Riso, R., Gaill, F., Laubier, L., Toulmond, A.: Biology and ecology of the "Pompeii worm" (*Alvinella pompejana* Desbruyères and Laubier), a normal dweller of an extreme deep-sea environment: A synthesis of current knowledge and recent developments. Deep-Sea Res. II *45:* 383-422, 1998.
2. Pereda, A., **O'Brien, J.**, Nagy, J.I., Smith, F., Bukauskas, F., Davidson, K.G.V., Kamasawa, N., Yasumura, T., and Rash, J.E. Short-range functional interaction between connexin35 and neighboring chemical synapses. Cell Commun. Adhes. 10: 419-423, 2003. PMCID: PMC1803252
3. Massey, S.C., O'Brien, J.J., Trexler, E.B., Li, W., Keung, J.W., Mills, S.L., and **O'Brien, J.** Multiple neuronal connexins in the mammalian retina. Cell Commun. Adhes. 10: 425-430, 2003.

4. **O'Brien, J.** The ever-changing electrical synapse. Current Opinion in Neurobiology 29:64-72, 2014.

Book Chapters

1. Vetter, R.D., Matrai, P.A., Javor, B., and **O'Brien, J.**: Reduced sulfur compounds in the marine environment: analysis by HPLC. ACS Symposium Series 393: 243-261, 1989.
2. Block, B.A., **O'Brien, J.**, and Franck, J.P.C.: The role of ryanodine receptor isoforms in the structure and function of the vertebrate triad. Soc. Gen. Physiol. Ser. 51: 47-65, 1996.
3. Li, C., **O'Brien, J.**, Al-Ubaidi, M.R. and Naash, M.I.: Organization of the chicken and *Xenopus* peripherin/*rds* gene. In New Insights into Retinal Degenerative Diseases. Eds. R.E. Anderson, M.M. LaVail, and J.G. Hollyfield. Alan R. Liss (New York). P269-277, 2001.
4. Li, H., and **O'Brien, J.**: Regulation of gap junctional coupling in photoreceptors. In Photoreceptors: Physiology, Types and Abnormalities. Eds. E. Akutagawa and K. Ozaki. Nova Science (Hauppauge, NY). 2012.
5. **O'Brien, J.**: Regulation of electrical synaptic plasticity in the retina by G-protein coupled receptors. In G protein Signaling Mechanisms in the Retina. Eds. K. Martemyanov and A.P. Sampath. Springer Life Science (New York). In press.

Manuscripts in Preparation or Submitted

1. Wang, Y., Kothmann, W.W., and **O'Brien, J.** Light-dependent modulation of connexin 35 phosphorylation in zebrafish retina. Manuscript in preparation.
2. Miller, C.S., Kothmann, W.W., O'Brien, J.J., and **O'Brien, J.** Highly coupled bistratified amacrine cells of the zebrafish retina. Manuscript in preparation.
3. Wang, H.Y., Mitchell, C.K., and **O'Brien, J.** Two-color fluorescent analysis of Connexin 36 turnover: relationship to functional plasticity. Manuscript submitted for publication.

Abstracts/Conference presentations in last 5 years (From 86)

1. Kothmann, W.W., Trexler, E.B., Li, W., Massey, S.C., and **O'Brien, J.** Presynaptic activity drives increased phosphorylation of connexin 36 in AII amacrine cells. Invest. Ophthalmol. Vis. Sci. 51: ARVO E-abstract 1208, 2010.
2. Li, H., Wang, S.W., Ribelayga, C., and **O'Brien, J.** Connexin36 phosphorylation is regulated by dopamine and adenosine in mouse photoreceptors. Invest. Ophthalmol. Vis. Sci. 51: ARVO E-abstract 6427, 2010.
3. Kothmann, W.W., Trexler, E.B., Li, W., Massey, S.C., and **O'Brien, J.** Signaling mechanisms that control gap junctional coupling between AII amacrine cells. 2010 FASEB conference on Retinal Neurobiology and Visual Processing.
4. **O'Brien, J.**, Ribelayga, C., Wang, S.W., and Li, H. Photoreceptor coupling is controlled by opposing actions of dopamine and adenosine receptors. 2010 FASEB conference on Retinal Neurobiology and Visual Processing.
5. Rash, J.E., Kamasawa, N., Nannapaneni, S., Davidson, K.G.V., Flores, C., Dicaro, A., Yasamura, T., **O'Brien, J.**, Nagy, J.I., Pereda, A. Neuronal gap junctions in goldfish hindbrain are primarily or

exclusively at glutamatergic mixed synapses and are heterotypic, with Cx35 in axon terminals and Cx34.7 in somata and dendrites. Program No. 42.3/G57. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010.

6. Rash, J.E., Kamasawa, N., Davidson, K., Yasumura, T., **O'Brien, J.**, Nagy, J.I., Pereda, A.E. Heterotypic Cx35/Cx34.7 coupling at rectifying gap junctions at goldfish giant club ending/Mauthner cell synapses demonstrated by double-replica FRIL. Mol. Biol. Cell 21(24): 4299. The American Society for Cell Biology 2010 Annual Meeting. Program #2225.
7. Marshak, D.W., Vila, A., Mills, S.L., Hoshi, H., Satoh, H., MacLeish, P.R., Marshak, D.R., **O'Brien, J.** Histamine receptors of cones and horizontal cells in macaque retina. Invest. Ophthalmol. Vis. Sci. 52: ARVO E-abstract 4112, 2011.
8. Zhang, Z., Liu, X., Li, H., **O'Brien, J.**, Ribelayga, C.P. Circadian clock control of connexin 36 phosphorylation in mouse retinal photoreceptors. Invest. Ophthalmol. Vis. Sci. 52: ARVO E-abstract 5282, 2011.
9. Kothmann, W.W., Trexler, E.B., Whitaker, C.M., Li, W., Massey, S.C., and **O'Brien, J.** Electrical synaptic plasticity in retinal AII amacrine cells is regulated by bipolar cell activity and closely associated non-synaptic NMDA receptors. Proceedings of the 2011 International Gap Junction Conference. Platform session 6 and poster 13.21.
10. Rash, J.E., Yasumura, T., Davidson, K., Moses, M.H., DiCaro, A.V., **O'Brien, J.**, Nagy, J.I., Pereda, A.E., Kamasawa, N. Distinguishing bi-homotypic vs. heterotypic gap junctions and homomeric vs. heteromeric connexons requires matched FRIL double-replicas. Proceedings of the 2011 International Gap Junction Conference. Poster 4.14.
11. Li, H., Wang, S.W., and **O'Brien, J.** Regulation of photoreceptor gap junction coupling by dopamine and adenosine. Mol. Biol. Cell 22 (suppl): Abstract # 381, 2011.
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SIGNIFICANT RESEARCH FINDINGS

O'Brien, J., G. Meissner and B.A. Block (1993). The fastest contracting skeletal muscles of non-mammalian vertebrates express only one isoform of the ryanodine receptor. Biophys. J. *65(12):* 2418-2427.

O'Brien, J., H.H. Valdivia and B.A. Block (1995). Physiological differences between the α and β ryanodine receptors of fish skeletal muscle. Biophys. J. *68(2):* 471-482.

Block, B.A., J. O'Brien and J.P.C. Franck (1996). Soc. Gen. Physiol. Ser. *51:* 47-65.

These papers discuss findings concerning the twin pair of calcium release channel (ryanodine receptor) isoforms in skeletal muscle. These channels reside, apparently side by side, in the sarcoplasmic reticulum and provide the calcium signal that activates muscle contraction. O'Brien et al., 1993 was the first study to demonstrate that the isoform distribution varied in different muscles and was related to muscle twitch speed. In O'Brien et al., 1995, we characterized several properties of the two channel isoforms, showing that the α isoform (now called RyR1) was more sensitive to calcium-activation and far more sensitive to calcium-inactivation than was the ß isoform (RyR3). Similar properties have subsequently been confirmed in mammalian RyR1 and RyR3. These results are reviewed and additional new data presented in Block et al., 1996. Our hypothesis is that rapid inactivation of the RyR1 isoform

by high calcium permits this isoform to operate in muscle at high twitch frequencies. Expression of the RyR3 isoform is expected to prolong the inactivation time of calcium release. These characteristics of excitation-contraction coupling are especially relevant because mammalian adult skeletal muscles express RyR1 almost exclusively, while both RyR1 and RyR3 are expressed during development. It is still unclear whether downregulation of RyR3 occurs through a requirement for high twitch frequencies or for some other reason. However, recent studies of knockout mice have shown that RyR1 is required for postnatal survival while RyR3 is not, in keeping with the observation that only RyR1 can support the direct mechanical form of EC coupling found in skeletal muscle.

O'Brien, J., M.R. Al-Ubaidi and H. Ripps (1996). Connexin 35: A gap junctional protein expressed preferentially in the skate retina. Mol. Biol. Cell *7:* 233-243.

White, T.W., M.R. Deans, J. O'Brien, M.R. Al-Ubaidi, D.A. Goodenough, H. Ripps, and R. Bruzzone (1999). Functional characteristics of skate connexin35, a member of the γ subfamily of connexins expressed in the vertebrate retina. Eur. J. Neurosci. *11(6):* 1883-1890.

Gap junctions are critical components of retinal circuitry but numerous attempts to identify the connexin proteins that form gap junctions in neurons had failed. O'Brien et al., 1996 reported the first cloning of a connexin from the retina, and showed that it was significantly different from all previously cloned connexins. Our expression studies (White et al., 1999) showed that this connexin exhibits unique properties that probably adapt it to function in neurons. The finding of a new connexin expressed in neuronal tissues has attracted a large number of laboratories to work on this protein.

O'Brien, J., R. Bruzzone, T.W. White, M.R. Al-Ubaidi, and H. Ripps (1998). Cloning and expression of two related connexins from the perch retina define a new subgroup of the connexin family. J. Neurosci. *18 (19):* 7625-7637.

Several very significant findings are presented in this study. Firstly, experiments with new antibodies to connexin 35 (Cx35) revealed expression of this connexin in many types of retinal neurons, confirming our suspicions that this is a neuronal connexin. This was the first demonstration that a specific connexin type is widely expressed in retinal neurons. Furthermore, significant expression was also detected in the brain, suggesting that many central nervous system neurons may use this connexin. Secondly, Cx34.7, a close relative of Cx35 and yet another new connexin, was also discovered in perch retina and brain. This showed that the diversity of physiological properties observed in gap junctions among retinal neurons is based, at least in part, upon a diversity of connexin types. Our expression studies showed differences in gating and conductance of the channels, and showed that they can interact to form heterotypic channels that may have unique properties. Perhaps the most significant finding of this work stems from a rigorous phylogenetic analysis of these connexins in relation to other members of the gene family. This analysis showed clearly that the new neuronal connexins belong to a unique branch of the connexin family. We consider it likely that the unique structural and functional properties of this subfamily of connexins are critical for their function in neurons. We furthermore hypothesized that these are likely candidate genes for certain neurological disorders; a suspicion confirmed when mutations in the gene were found to be linked to Juvenile Myoclonic Epilepsy.

Mills, S.L., O'Brien, J.J., Li, W., O'Brien, J., and Massey, S.C. (2001). Rod pathways in the mammalian retina utilize connexin 36. J. Comp. Neurol. 436: 336-350.

Pereda, A., O'Brien, J., Nagy, J.I., Bukauskas, F., Davidson, K.G.V., Yasumura, T., and Rash, J.E. (2003). Connexin35 mediates electrical transmission at mixed synapses on Mauthner cells. J. Neurosci. 23(20): 7489-7503.

O'Brien, J., Nguyen, H.B., and Mills, S.L. Cone photoreceptors in bass retina use two connexins to mediate electrical coupling. J. Neurosci. 24(24): 5632-5642.

These studies provide the first identifications of specific connexin proteins at electrical synapses in defined neural circuits. In each case, several important physiological properties of the electrical synapses have been described. The most widely studied electrical synapses are those in the retinal AII amacrine cells and those between photoreceptors. We have found that both of these circuits utilize Cx35 or its mammalian orthologue Cx36. In Mills et al., 2001, we show that Cx36 is the primary connexin at AII amacrine to AII amacrine cell homologous gap junctions as well as to AII amacrine to cone ON bipolar cell heterologous gap junctions. Although in the same cell type, these gap junctions are regulated by different pathways: the dopamine D1 receptor/PKA pathway for AII-AII junctions, and the nitric oxide/PKG pathway for AII-cone bipolar gap junctions. Thus this connexin is a target for several regulatory pathways. In O'Brien et al., (2004) we find that fish cone photoreceptors use a different strategy. First, we show that Cx35 activity is regulated dynamically by PKA, in keeping with its role in amacrine cell gap junctions, although Cx34.7 gap junctions were not affected. Furthermore, we have found that bass cones express both Cx35 and Cx34.7 in different gap junctions. Thus different functional regulation is achieved by expression of different connexins. The most surprising revelation is that both Cx35 and Cx34.7 appear to be involved in gap junctions between cones. Cx34.7 gap junctions have very close proximity to the synaptic zones, and we hypothesize that these gap junctions constitute a coupling pathway with distinct regulatory properties. Finally, in Pereda et al., 2003, we find that Cx35 is the primary connexin at another well-studied mixed synapse in the central nervous system. This Mauthner cell gap junction shows a great deal of activity-dependent plasticity, including short-term potentiation. These findings show that Cx35 is at the heart of many dynamic processes at electrical synapses, and suggests that the huge number of electrical synapses throughout the central nervous system that utilize Cx35 have great potential for plasticity.

Ouyang, X., Winbow, V.W., Patel, L.S., Burr, G.S., Mitchell, C.K., and **O'Brien, J.** Protein kinase A mediates reversible regulation of electrical synapses containing connexin35 through a complex pathway. Mol. Brain Res. 135(1-2): 1-11, 2005.

Patel, L.S., Mitchell, C.K., Dubinsky, W.P., and **O'Brien, J.** Regulation of gap junction coupling through the neuronal connexin Cx35 by nitric oxide and cGMP. Cell Commun. Adhes. 13(1-2): 41-54, 2006.

Ouyang et al. is the first study to comprehensively reveal the molecular mechanisms of regulation of a neuronal gap junction by a signaling pathway. We characterized the regulation of Cx35 gap junctional coupling by PKA, a key kinase that effects cAMP signaling. We found that Cx35 is regulated by concomitant phosphorylation on two key residues, one in the intracellular loop and one in the C-terminal tail. We also discovered that signaling through the very last few amino acids of the C-terminal tail acts as a molecular switch that can reverse the normal response to phosphorylation on the two major sites. This is the first example of such a switch in a gap junction and helps to explain the diversity of regulatory responses observed in different neurons expressing this connexin. Patel et al. extended this type of analysis to the nitric oxide signaling pathway. In this study we found that NO signaling is more complex than PKA signaling. Some of the PKG phosphorylation sites found had little regulatory function, and others overlapped the regulatory PKA sites. Furthermore, we found that NO signaling is routed through the PKA pathway in HeLa cells. These studies establish the molecular basis for regulating communication through electrical synapses in the central nervous system.

Kothmann, W.W.**,** X. Li, Burr, G.S., and **O'Brien, J.** Connexin35/36 is phosphorylated at regulatory sites in the retina. Vis. Neurosci. 24(3): 363-375, 2007.

This manuscript follows up on our study of the regulation of Cx35 gap junctions by phosphorylation. We developed phospho-specific antibodies targeted toward the two key regulatory sites of Cx35. We found that both of these sites are indeed phosphorylated in the retina. However, phosphorylation is not uniform throughout the retina. Cx35 gap junctions in close proximity display strikingly different phosphorylation states, suggesting that phosphorylation is controlled at the level of individual cells or perhaps individual gap junctions. Furthermore, some populations of gap junctions display large changes in phosphorylation state in response to light adaptation. The changes suggest that coupling through these gap junctions changes as the retina adapts to light. These antibodies provide a tool to predict the coupled state of gap junctions in circuits that are not readily studied physiologically.

Kothmann, W.W., Massey, S.C., and **O'Brien, J.** Dopamine-stimulated dephosphorylation of connexin 36 mediates AII amacrine cell uncoupling. J. Neurosci. 29(48): 14903-14911, 2009.

Li, H., Chuang, A.Z., and **O'Brien, J**. Photoreceptor coupling is controlled by connexin 35 phosphorylation in zebrafish retina. J. Neurosci. 29(48): 15178-15186, 2009.

These manuscripts show the first correlations of gap junction phosphorylation with functional coupling in identified neural networks. In both studies, we have found that Cx35 or Cx36 phosphorylation is directly and quantitatively correlated with coupling. Thus we have developed the only biochemical tools currently known (i.e. phospho-Cx35/36 specific antibodies) that quantitatively report the functional state of an electrical synapse. In the rabbit AII amacrine cell network, we have elucidated the signaling pathway by which dopamine D1 receptors uncouple gap junctions. This works by protein kinase A activation of protein phosphatase 2A, and de-phosphorylation of Cx36. We have further seen that the phosphorylation states, and hence the functional states, of gap junctions within a single cell are controlled independently. This implies local control of coupling and provides the substrate for activity-dependent control of coupling analogous to LTP in chemical synapses. In the photoreceptor network, we have found that regulation by protein kinase A activity is diametrically opposite to that in the AII amacrine cell network. PKA activity directly increases Cx35 phosphorylation and coupling, and is robustly controlled by diurnal light cycles. Thus these two studies show that each neural network can assemble signaling pathways that achieve appropriate regulation of coupling, even if these are diametrically opposing responses to the same second messenger (e.g cAMP and PKA activity).

Kothmann, W.W., Trexler, E.B., Whitaker, C.M., Li, W., Massey, S.C., and **O'Brien, J.** Nonsynaptic NMDA receptors mediate activity-dependent plasticity of gap junctional coupling in the AII amacrine cell network. J. Neurosci. 32(20): 6747-6759, 2012.

This manuscript demonstrates the novel finding that non-synaptic NMDA receptors on AII amacrine cells are directly associated with gap junctions and serve the apparently dedicated purpose to regulate gap junctional coupling. This confers a form of activity-dependent plasticity on electrical synapses that is likely to be widespread throughout the central nervous system. This activity-dependent plasticity employs a Ca^{2+}, CaMKII-mediated pathway to phosphorylate Cx36 gap junctions independently of the cAMP-PKA-PP2A pathway that de-phosphorylates these gap junctions. This functional segregation of signaling pathways is responsible for the biphasic (inverted U-shaped) adaptation curve that characterizes the changes of AII amacrine cell coupling in response to light adaptation.

Li, H., Zhang, Z., Blackburn, M.R., Wang, S.W., Ribelayga, C.P. and **O'Brien, J.** Adenosine and dopamine receptors co-regulate photoreceptor coupling via gap junction phosphorylation in mouse retina. J. Neurosci. 33(7): 3135-3150, 2013.

Li, H., Chuang, A.Z., and **O'Brien, J.** Regulation of photoreceptor gap junction phosphorylation by adenosine in zebrafish retina. Vis. Neurosci. 31(3): 237-243, 2014.

These manuscripts demonstrate that photoreceptor gap junction coupling is controlled by opposing actions of dopamine and adenosine receptors that regulate the level of connexin phosphorylation. The opposing actions of these receptors impose a fine level of control on photoreceptor coupling by comparing levels of extracellular signals dopamine and adenosine that respectively signal light and dark. By using opposing forces, changes in coupling are both precise and strong, resulting in a large change in coupling at appropriate times.

Rash, J.E., Curti, S., Davidson, K.G.V., Kamasawa, N., Nannapaneni, S., Flores, C.E., Yasumura, T., **O'Brien, J.**, Lynn, B.D., Nagy, J.I., and Pereda, A.E. Molecular and functional asymmetry at a vertebrate electrical synapse. Neuron 79(5): 957-969, 2013.

This manuscript details the remarkable observation that fish Mauthner cell electrical synapses use a heterotypic pairing of two different connexin proteins to form the electrical synapses that connect auditory nerve afferents to the Mauthner cell, a large motor neuron. These synapses are a unique mixed synapse (containing both electrical and glutamatergic chemical synapses) that plays a critical role in triggering the escape reflexes in most vertebrates. The heterotypic pairing of Cx35 and Cx34.7 allows for partial rectification of electrical signals at this synapse, optimizing signal flow to reinforce synaptic transmission.