BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Kevin J Staley	Professor, Neurology and Pediatrics
eRA COMMONS USER NAME Staleyk	

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
Loyola Marymount University, Los Angeles, CA	B.S.	1979	Physics
Univ. of California. San Diego, CA	M.D.	1984	Medicine

A. Personal Statement: **A. Personal Statement:** I have been investigating the pathophysiology of neonatal seizures since my original career development award in 1991. After two decades of research I am delighted that our more recent basic science findings regarding bumetanide-sensitive neuronal chloride transport have been translated into a multicenter therapeutic clinical trial for neonatal seizures (R01NS066929, ClinicalTrials.Gov # NCT00830531). I look forward to applying the tools we are developing in our BRAIN consortium to the study of GABA signaling and neuronal chloride homeostasis in health and after acute brain injury, and to continuing our investigations into the mechanisms and treatments of chronic epilepsy.

B. Position and Honors

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1981	Scripps Institute of Oceanography	NIH Summer Research Fellowship with Ted Bullock
1984-86	Univ. of California. San Diego	Pediatric Resident
1986-87	University of Colorado	Adult Neurology Resident
1987-89	Univ. of California. San Diego	Child Neurology Resident
1988-89	Univ. of California. San Diego	Instructor, Pediatrics
1989-91	Stanford University	Dana Research Fellowship with David Prince and Istvan Mody
1989-91	Stanford University	Instructor, Neurology
1991-97	University of Colorado	Assistant Professor, Neurology and Pediatrics
1997-04	University of Colorado	Associate Professor, Pediatrics and Neurology
2004-06	University of Colorado	Professor, Pediatrics and Neurology
2006-	Massachusetts General Hospital	Neurologist and Chief, Pediatric Neurology
2007-	Harvard Medical School	Professor of Neurology

HONORS & PUBLIC ADVISORY COMMITTEES

1979	Alpha Sigma Nu (Jesuit Honor Society)
1979	Sigma Pi Sigma (Physics Honor Society)
1989	UCSD Neuroscience Department, Pediatric Neurology Division, Standard of Excellence Award
1996-07	NIH CSR NLS-2 / BDCN2 / CNNT
2002-4	American Epilepsy Society: Chair, Investigator's Workshop Committee
2004-6	Epilepsy Foundation of America: Chair, Fellowship Training Committee
2004-10	*Journal of Neuroscience*: Associate Editor
2006	Gordon Research Conference: Co-Chair, Mechanisms of Epilepsy and Neuronal Synchronization
2007	University of Colorado: St. Geme prize, best mentored student research (Audrey Brumback)
2008-10	American Epilepsy Society: Chair, Research and Training Committee
2009-12	Epilepsy Foundation of America: Chair, Research Council
2009-12	Society for Neuroscience: Program Committee, Neurobiology of Disease
2012-14	American Neurological Association: co-Chair, Epilepsy Special Interest Group

2011- Recent NIH review activity:
Study section chair: ZNS1 SRB-B 32 (Epilepsy EUREKA), ZNS1 SRB-B 29
Study section participation: ZNS1 SRB-B 27, ZNS1 BDCN-J, CNNT
2013-5 NINDS Board of Scientific Counselors
2013 NIH NINDS Curing Epilepsy Conference: co-chair
2013 World Organization for the Neurobiology of Epilepsy (WONOEP) Conference: co-chair
2015-7 Chair, NIH NINDS Board of Scientific Counselors
2015-22 Javits Neuroscience Investigator Award (R37)

C. Contributions to Science

1. Activity-dependent changes in the $GABA_A$ reversal potential. This important mechanism of activity-dependent disinhibition was a puzzle for almost two decades. One reason the puzzle was difficult to solve was the presence of a large, non-inactivating chloride conductance that activated at membrane potentials < E_{Cl}, permitting Cl^- efflux until E_{Cl} = RMP. This greatly complicated the interpretation of voltage clamp experiments. Once we understood this conductance (1994) it was possible to use voltage clamp studies of Cl^- transport to show that sustained activation of GABA receptors could admit Cl^- at a rate that transiently exceeded export capacity (1995). In small structures such as dendrites, the resultant chloride accumulation shifted E_{Cl} to the membrane potential. Under these conditions, net ion flux through the $GABA_A$ channel is carried by HCO_3^-, normally a minor component of the current. E_{HCO3} is stabilized near 0 mV by CO_2 diffusion across the membrane, carbonic anhydrase, and pH buffering, so E_{GABA} is very strongly depolarized. We described the kinetics of Cl transport more completely in the 1999 *J Physiol* paper. New technology (2 photon imaging of transgenic Cl-sensitive fluorophores) has confirmed that preictal accumulation of Cl is an important mechanism of transient, activity-dependent disinhibition and ictogenesis (Lillis et al. 2012). We have created new Cl reporters (BRAIN U01) to study Cl kinetics at the level of dendrites and GABA receptors (this proposal).

1. Staley, KJ The role of an inwardly rectifying chloride conductance in postsynaptic inhibition *J. Neurophysiology*. 72:273-284, 1994

2. Staley KJ Soldo B and Proctor W. Ionic mechanisms of neuronal excitation by inhibitory GABAA receptors *Science* 269:977-981, 1995.

3. Staley KJ and Proctor W. Modulation of mammalian dendritic GABA receptor function by the kinetics of Cl- and HCO3-transport. *J. Physiol. (Lond).* 519(Pt 3):693-712, 1999

4. Lillis KP, Kramer MA, Mert J, Staley KJ, White JA. Pyramidal cells accumulate chloride at seizure onset. *Neurobiol Dis* 47:358-66, 2012

2. Bumetanide for neonatal seizures: The 1989 description of depolarizing GABA responses in early development by Cherubini and Ben Ari raised the possibility that neonatal seizures do not respond to anticonvulsants because of a depolarized E_{GABA}. In 1992 we loaded adult neurons with Cl via the recording pipette to show that in the presence of a sufficiently depolarized E_{GABA}, even anesthetic concentrations of allosteric $GABA_A$ anticonvulsants (pentobarbital) were ineffective. In 2005 we used the diuretic bumetanide to inhibit NKCC1, a chloride-accumulating cotransporter expressed in developing neurons, shifted E_{GABA} to negative values and inhibited kainate-induced seizures rat pups in vivo. In 2008 we showed that the bumetanide shift in E_{GABA} improved the efficacy of GABAergic anticonvulsants (phenobarbital). In 2009 we demonstrated a mechanism underlying electroclinical dissociation, the widely observed ICU EEG phenomenon of nonconvulsive EEG seizure activity. These papers helped us structure the clinical bumetanide study (i.e. incorporating long-term EEG monitoring of all enrolled patients, and adding vs substituting bumetanide and phenobarbital treatments). In 2010 we used 2-photon microscopy and Cl-sensitive fluorophores to demonstrate that a reduction in cytoplasmic Cl was the basis of the anticonvulsant effects of NKCC1 inhibition. Together, these findings comprise the “bench” foundation for an ongoing, bench-to-bedside, NIH-funded, multicenter trial of bumetanide for the treatment of neonatal seizures (Clinicaltrials.gov #NCT00830531).

1. Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire EJ, Jensen FE, and Staley KJ. NKCC1 transporter facilitates seizures in the developing brain. *Nature Medicine*, 11:1205-13,

2005

2. Dzhala V Brumback A and Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Ann Neurol* 63:222-235, 2008

3. Glykys J, Dzhal V, Kuchibhotla KV, Feng G, Kuner T, Augustine G, Bacskai BJ, Staley KJ. Differences in cortical vs. subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. *Neuron* 63:657-72, 2009.

4. Dzhala V, Kuchibhotla KV, Glykys J, Kahle KT, Swiercz W, Feng G, Kuner T, Augustine G, Bacskai BJ, Staley KJ Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures *J Neurosci* 30:11745-61, 2010.

3. Chloride microdomains: Continuing to do bench research in support of the bumetanide trial, we discovered a number of discrepancies between current theories regarding the means by which E_{GABA} is determined (i.e., by equilibrative transporters) and findings such as the minimal effect of transport inhibition on E_{GABA} in healthy neurons. We first addressed artifactual determinants of E_{GABA}, such as trauma to the superficial neurons in acute brain slice preparations (Dzhala et al. 2012). Next we considered that the majority of cytoplasmic anions do not permeate the GABA receptor, and calculated the effects of the corresponding Donnan equilibrium on E_{GABA}. However, this did not adequately explain the Cl distribution, nor E_{GABA}. We added the water transport capacity of Cl cotransporters, the otherwise poor water permeability of neuronal membranes (which have no aquaporins), and cytoplasmic idiogenic osmoles (e.g. taurine) that balance osmotic gradients. With these additional elements, the wide inter-neuronal and intra-neuronal variance in E_{GABA} could be satisfactorily explained (Delpire and Staley 2014; Glykys et al. 2014). An intriguing implication of the role of impermeant, macromolecular polyanions (such as polysulfated glycosaminoglycans in the extracellular space, and polyglutamylated microtubules in the cytoplasm) is the existence of Cl microdomains. These could impart a unique E_{GABA} at each individual $GABA_A$ synapse. Roger Tsien has demonstrated exceptionally slow turnover of extracellular glycosaminoglycans (SFN abstract 785.16, 2014), so Cl microdomains could comprise a form of long-term information storage. We were awarded a BRAIN grant to create the tools to look for these Cl microdomains, in collaboration with Robert Macdonald, George Augustine, and Brian Bacskai (U01 MH106013). We will use these tools in the current proposal to test the microdomain hypothesis.

1. Dzhala V, Valeeva G, Glykys J, Khazipov R, and Staley KJ. Traumatic alterations in GABA signaling disrupt hippocampal network activity in the developing brain, *J Neurosci* 32:4017-31, 2012

2. Glykys J, Dzhala V, Egawa K, Balena T, Saponjian T, Kuchibhotla KV, Bacskai BJ, Kahle KT, Zeuthen T, Staley KJ Local Impermeant Anions Establish the Neuronal Chloride Concentration *Science* 343(6171):670-5, 2014

3. Delpire E, Staley KJ. Novel determinants of the neuronal Cl(-) concentration*. J Physiol*. 2014 Oct 1;592(Pt 19):4099-114.

4. Staley K. Molecular mechanisms of epilepsy *Nat Neurosci* 2015.

4. Origin of synchronous activity in neural networks: In 1995 we hoped to cure epilepsy by blocking activity-dependent changes in E_{GABA} (see #1). However, this had minimal effects on periodic population bursting induced by convulsants in area CA3 of acute brain slice preparations. We studied the determinants of the timing of CA3 bursts and came to the surprising conclusion that this was largely independent of GABA, and was instead determined by short-term (Staley et al. 1998) and long-term (Bains et al. 1999) determinants of the strength of glutamatergic synapses that link the neurons in the network. When we discovered that the organotypic slice culture developed spontaneous electrographic seizures in the absence of convulsants, we switched to this preparation as a model of post-traumatic epileptogenesis. We demonstrated the stochastic nature of network activation during epileptic spike activity (i.e. no pacemaker cells), and the simplification of network activation by convulsants that block the outputs of inhibitory networks (Sabolek et al. 2012). Two advantages of the organotypic slice are that there are no inputs that might trigger seizures, and the slice cultures can be imaged continuously for weeks at a time. We are exploiting these advantages to study epileptogenesis and ictal cell death at high spatial and temporal resolution (Lillis et al. 2015).

1. Staley KJ, Longacher M, Bains, J, and Yee A. Presynaptic modulation of CA3 network activity. *Nature Neurosci.* 1:331,1998

2. Bains JS, Longacher JM and Staley KJ. Reciprocal interactions between CA3 network activity and strength of recurrent collateral synapses. *Nature Neurosci.* 2:720-6, 1999

3. Sabolek HR, Swiercz WB, Lillis K, Cash SS, Huberfeld G, Zhao G, Ste. Marie L, Clemenceau S, Barsh G, Miles R, Staley KJ: A candidate mechanism underlying the variance of interictal spike propagation. *J Neurosci* 32:3009-3021, 2012.

4. Lillis K, Wang Z, Berdichevsky E, Mail M, and Staley KJ. Evolution of network synchronization during early epileptogenesis parallels synaptic circuit alteration. *J Neurosci* in press 2015.

5. EEG studies of the natural history of epilepsy: To test the predictions of our in vitro findings regarding the activation of epileptic networks in vivo, we collaborated with Ed Dudek, who had developed expertise in the kainate and pilocarpine models of acquired epilepsy. We hoped to demonstrate that long-term weakening of synaptic strength would cure epilepsy (it did not), but testing whether epilepsy is cured requires the ability to detect a seizure frequency of 0, i.e. an infinitely long inter-seizure interval. This required continuous EEG monitoring and accurate, minimally supervised EEG analysis for the detection of very rare seizures in months-long EEG recordings. We published the original methods papers in 2006 (White et al.), and used this technology to describe the surprisingly gradual onset of acquired epilepsy (Williams et al. 2009), seizure clustering (Kadam et al. 2010) and the potential importance of interictal spikes as a predictor of epilepsy after brain injury (White et al. 2010). We are currently using these techniques to execute a large-scale study to test whether interictal spikes precede acquired epilepsy after more clinically relevant brain injuries including trauma and stroke (R01 NS086364).

1. White AM, Williams PA, Ferraro DJ, Clark S, Kadam SD, Dudek FE, and Staley KJ. Efficient Unsupervised Algorithms for the Detection of Seizures in Continuous EEG Recordings from Rats after Brain Injury. *J Neurosci Methods*, 15;152:255-66, 2006.

2. Williams PA, White AM, Clark S, Ferraro DJ, Swiercz W, Staley KJ, Dudek FE. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. *J Neurosci*. 18;29(7):2103-12, 2009.

3. Kadam S, White A, Staley K, and Dudek E. Continuous electroencephalographic monitoring with radio-telemetry in a rat model of perinatal hypoxia-ischemia reveals progressive post-stroke epilepsy *J Neurosci* 30(1):404-15, 2010

4. White A, Williams PA, Hellier J, Clark S, Dudek FE and Staley KJ. EEG spike activity precedes epilepsy after kainate-induced status epilepticus. *Epilepsia* 51(3):371-83, 2010.

Public bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/kevin.staley.1/bibliography/41161262/public/?sort=date&direction=ascending

D. Research Support
ACTIVE

5R01NS40109-14 (Staley) 01/15/11-12/31/15, renewal in review
NIH/NINDS
Impact of Neuronal Chloride transport on treatment of seizures
The goal of this grant is to develop new treatments for neonatal seizures based on the unique aspects of neuronal chloride transport at this developmental stage.

5R01NS066929-05 (Soul) 08/02/10-06/30/15
NIH/NINDS
Pilot Trial of Bumetanide.
The goal of this grant is Phase II trial of a compound we initially identified in the lab as potentially beneficial for neonatal seizure therapy.
Role: PI of Subcontract to Children's Hospital.

R37 NS077908-04 (Staley) 07/01/15-06/30/22
NIH/NINDS
Mechanisms of neuronal death during epileptogenesis.

The goal of this research is to understand mechanisms of ictal neuronal death.

K12NS066225-04 (Staley) 09/01/11-06/30/16
NIH/NIDS
Pediatric Neurology Physician Scientist Program.
This grant will support research training of pediatric neurology junior faculty members.
The goal of this grant is training new physician scientists in pediatric neurology.

5R01NS034700-22 (Staley) 06/01/12-02/28/17
NIH/NINDS
Development of epileptic circuits.
The goal of this grant is to understand the circuit alterations that produce epileptic neural networks.

5R01NS086364-02 (Staley) 09/01/13-05/31/18
NIH/NINDS
Biomarkers for epileptogenesis after brain injury
The goal of this grant is preclinical validation of electrographic biomarkers after clinically relevant experimental brain injuries.

1U01MH106013-01 (Staley) 09/26/14-06/30/17
NIH/NINDS
Mapping neuronal chloride microdomains
The goal of this NIH BRAIN tool-creation U01 project is to develop the ratiometric Cl^- fluorophores that will enable experiments that test for neuronal Cl^- microdomains. My role in the U01 project is to test the function of the created fluorophores in vitro. This U01 project does not fund experiments to test for or map neuronal Cl^- microdomains as is proposed here, so there is no scientific overlap with R01NS40109.

U02NS077179 (Cudkowicz) 09/01/11-08/31/18
NIH/NINDS
Clinical Coordinating Center for the Network of Excellence in Neuroscience
The objective of the network of excellence in Neuroscience Clinical Trials initiative is to rapidly and efficiently translate advances in neurosciences into treatments for people with neurological disorders. My role in this project is to lead NeuroNEXT protocol working groups that assist PIs in the development of clinical trial protocols in pediatric neurology.
Role: Investigator

COMPLETED:

5R21NS072258-02 (Staley) 01/15/11-12/31/14**
NIH
Moderate-throughput screening for anti-epileptogenic drugs.
This project used new in vitro technologies to screen for drugs with antiepileptogenic activity. We screened 500 drug / concentration / ionic conditions, and are preparing the results for publication.

Mapping the escape from inhibition.
The goal of this nonrenewable EUREKA project was to understand the wiring of interneurons and their contributions to neural network operation. We discovered the variance of GABA signaling during this project, leading to Dzhala et al. 2012, the Glykys et al. Science paper, the BRAIN U01 project, and the experiments proposed in the current application.

5R01NS74772-04 (Staley) 08/01/11-04/30/15
NIH/NINDS