Research Interest

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Neurophysiology laboratory and the techniques employed

I joined Auburn University in 2001 and established a research laboratory with the start up funds of $ 50, 000 and developed it into a neurophysiology laboratory that is capable of performing single channel, intracellular, extracellular, and in vivo electrophysiological recordings along with molecular, biochemical and behavioral methodologies.

One of the ***unique techniques that we developed in 2001*** was to directly record single channel currents of synaptic neurotransmitter receptors and this work was latter published *(Methods. Enzymol. 2006, 417:80-90)*. There are currently no other techniques available to directly measure the functionality of synaptic receptors, which is a critical measure of synaptic transmission. We use an upstream approach to investigate how modifications in the electrical properties (Channel open probability, conductance, dwell time distribution and burst activity) of single synaptic glutamate (AMPA & NMDA) receptors will alter the electrical properties of the neurons in; animal and tissue models of neurodegeneration *(J. Neuropathol. Exp. Neurol. 2007, 66:779-788; Exp. Neurol. 2008, 214:55-61),* animal models of diabetes and prenatal alcohol exposure *(Neurobiol. Dis. 2007, 26:696-706: Neurobiol. Dis. 2008, 29:81-91).* When electrical properties of group of neurons are altered, this may lead to dysfunction of specific regions of the brain and in turn can cause behavioral deficits. Currently, we have the technology available to investigate how modifications in the electrical properties of single synaptic glutamate receptors (synaptosomal recording) can lead to altered synaptic currents (whole cell patch clamp technique) which in turn may modify plasticity mechanisms (slice & in vivo electrophysiology) resulting in behavioral deficits (Morris Water Maze, Y-Maze, & object recognition) in animals models of Alzheimer's disease and diabetes. Collaborating with the new faculty members in our department with expertise in cutting edge molecular biology techniques, we have already begun to probe the intracellular signaling pathways that contribute to altered expression and modified electrical properties of synaptic glutamate receptors in animal models of diabetes and Alzheimer's disease. I describe below *two of the major projects* in our laboratory:

Insulin Resistance, Integrin linked kinase (ILK) and hippocampal synaptic plasticity:

One of our recent major research focuses is on insulin signaling, ILK, hippocampal synaptic plasticity and learning and memory. We have found that in an animal model of type-1 diabetes (STZ-diabetes), learning and memory deficits are due to altered functionality of synaptic AMPA receptors resulting from modified expression of synaptic molecules such as GluR1, NCAM-PSA and PSD 95. In this study, we showed that peripheral insulin deficiency causes deficits in hippocampal synaptic plasticity, at least, in part, due to altered insulin signaling in the brain. To investigate the down stream signaling mechanism resulting from insulin resistance leading to deficits in glutamatergic synaptic transmission causing learning and memory impairments, we have utilized the intracranial streptozotocin (ic-STZ) rodent model. In this model, intracerebroventricular injection of STZ will cause insulin resistance in the brain. The ic-STZ model is also considered as a sporadic model of Alzheimer's disease.

The activation of insulin receptors results in downstream activation of the phosphatidylinositide 3 kinase (PI3)/Akt kinase (Akt) pathway, an important signaling pathway for synaptic plasticity. Akt inactivates glycogen synthase kinase-3β (GSK-3β) by phosphorylation of the serine-9 residue. Furthermore, GSK-3β has been shown to coimmunoprecipitate with GluR1/2 AMPA receptor subunits and GSK-3β activity is essential for determining the direction of synaptic plasticity, with its inactivation being crucial for the induction of long-term potentiation (LTP), a cellular model for

memory. Recent evidence suggests that ILK may be the dominant kinase involved in GSK-3β inactivation in the hippocampus; however, little is known about its involvement in neuronal physiology. The current understanding of the ILK-GSK-3β pathway in synaptic physiology is limited to its role in regulating dendrite formation in developing hippocampal neuron. Since GSK-3β over activation is found in both AD and type-2 diabetes, we have investigated whether alterations in synaptic function correlate with changes in the ILK-GSK-3β pathway. *Our recent findings strongly suggest a central role for the ILK-GSK3-β pathway in synaptic dysfunction in the insulin resistant brain.* This synaptic disruption is *mainly mediated by altered expression and function of synaptic AMPA receptors*. If this is the case, inhibition of ILK or knocking down ILK expression in the hippocampus should cause similar effects. We are currently utilizing organotypic hippocampal cultures to test this hypothesis. Another recent finding from our laboratory is that the Alzheimer's peptide Ab_{1-42} decreased the ILK expression in hippocampal slices. We have previously shown that the Ab_{1-42} directly modulates AMPA receptor mediated glutamatergic synaptic transmission (Exp. Neurol. 2009, 210:7-13; Synapse 2009 61:367-374). We are currently investigating the role of ILK in insulin signaling and Alzheimer pathology. *This work is currently being developed into an NIH R01 for submission in October.*

Subunit specific modulation of NMDA receptors by PSA: implications for synaptic plasticity and learning. This project is carried out in collaboration with the laboratory of *Dr. Alexander Dityatev* of Italian Institute of Technology. We have previously shown that Neural Cell Adhesion Molecule associated polysialic acid (NCAM-PSA) potentiates AMPA receptor function *(J. Biol. Chem. 2004 279:47975-47984)* and inhibits NR2B containing extrasynaptic NMDA receptors *(J. Biol. Chem. 2006 281:34859-34869)*. In this project, we aim to demonstrate that impairment in LTP and learning in NCAM/PSA-deficient mice are rescued by suppressing extrasynaptic NR2B-NMDA receptors or by enhancing the activity of NR2A-NMDA receptors. If this is the case, ablation of *Ras-GRF1*, a mediator of NR2B signaling to p38 mitogen activated protein kinase (MAPK), or direct inhibition of p38 MAPK also can restore impaired LTP in brain slices lacking PSA/NCAM. We proposed that PSA carried by NCAM restrains NR2B/Ras-GRF1/p38 signaling and regulates synaptic plasticity and learning by properly balancing the transmission through NR2B- and NR2A-NMDA receptors. We are utilizing synaptosomal single channel recording and intracellular electrophysiology (to record extrasynaptic NMDA receptors), synaptic plasticity measures (LTP & LTD), two-photon imaging (to visualize calcium influx through extrasynaptic NMDA receptors in the absence of PSA), biochemical measures to detect p38MAPK signaling through extrasynaptic NMDA receptors, and behavioral experiments (fear conditioning) to demonstrate deficits in learning. Part of this project is submitted as an NSF grant that is currently being reviewed.

Below I describe some of the ***Research highlights of the laboratory:***

Our laboratory was the first to: i) develop a technique to directly measure the single channel properties of synaptic AMPA and NMDA receptors and demonstrate the interactive (cooperative) channel gating of synaptic AMPA receptors *(Methods. Enzymol. 2006, 417:80-90)*. ii) demonstrate the direct modulation of synaptic AMPA receptors by Alzheimer peptide Ab_{1-42}, iii) demonstrate the direct modulation of AMPA receptors by PSA and thereby establishing the neuroprotective role played by PSA (*J. Biol. Chem. 2004 279:47975-47984*), iv) demonstrate subunit and region specific modulation of NMDA receptors by NCAM-PSA (*J. Biol. Chem. 2006 281:34859-34869*), v) elucidate the molecular mechanism of memory loss in prenatal alcohol exposure *(Neurobiol. Dis. 2007, 26:696-706)* and identify a possible therapeutic option (*Neurobiol. Dis. 2008, 29:81-91).*