Alzheimer’s disease (AD) is a neurodegenerative disorder that affects millions worldwide. It is the leading cause of dementia, and is most commonly diagnosed in individuals older than 65.1 Although the initial cause and pathogenesis are not well understood, research has shown that classic hallmark foci found in the brains of AD individuals are cholinergic neuron degeneration, extracellular beta-amyloid plaques, and intracellular neurofibrillary tangles (NFT) composed of hyperphosphorylated Tau protein.2 Normally, Tau stabilizes microtubules and plays an important role in intracellular transport, but in AD these processes become disrupted.2

The hyperphosphorylated Tau state can be simulated in vitro by treating embryonic (E19) rat cortical neuronal cultures with okadaic acid, a phosphatase inhibitor. This primary culture system has been used previously to investigate various Tau phosphorylation pathways5-11 in this study, we used a novel immunosensor system, Gyros, to evaluate the effects of okadaic acid and various kinase inhibitors (AKT inhibitor: MK2 inhibitor; staurosporine; mTOR inhibitor: Torin1; PK8807305; PK-0241350; and GSK3 inhibitor PF-04820367) on Tau-phospho-Tau. We investigated Tau phosphorylation in vivo, the Tg4510 transgenic mouse model was used. This model expresses the P301L mutant human Tau allele (linked to hereditary tauopathies), where expression of the Tau variant leads to development of NFT, neuronal death, and memory impairment in the mice, which is consistent with the pathology observed in human AD patients11-12. In this study, we used Gyros and traditional Western Blot to evaluate the Tau-phospho-Tau levels in the brain homogenates of Tg4510 mice (wild type male and female, as well as bicuspid +/- male and female; n=10 for each category) at various time points: 3.5, 6.5, and 8 months. We have used Gyros to develop and optimize several rat and human Tau-phospho-Tau assays, including total Tau, AT270, AT8, AT100, AT180, pSer262, and PFP13. In this study, we first aimed to establish Gyros as a technology platform for in vitro applications. We then compared analysis of the Tg4510 brain homogenates using Gyros and Western Blot in order to validate the Tg4510 mouse model. Gyros allows for high-throughput analysis of low-volume in vivo or in vitro samples with a high level of sensitivity which is comparable to other immunoassays systems, critical parameters for drug discovery.

**RESULTS**

**Alzheimer’s Disease**

Alzheimer’s disease (AD) is a neurodegenerative disorder that affects millions worldwide. It is the leading cause of dementia, and is most commonly diagnosed in individuals older than 65.1 Although the initial cause and pathogenesis are not well understood, research has shown that classic hallmark foci found in the brains of AD individuals are cholinergic neuron degeneration, extracellular beta-amyloid plaques, and intracellular neurofibrillary tangles (NFT) composed of hyperphosphorylated Tau protein.2 Normally, Tau stabilizes microtubules and plays an important role in intracellular transport, but in AD these processes become disrupted.2

**Methods**

### Rat Cortical Neuron Preparation and Treatment

1. Rat cortical neurons were harvested from E18 embryos and plated in a 96 well plate, 5000 cells per well. 2. Cells were then cultured in hippocampal and cortical cell media (HCM) containing B27 (Invitrogen) and GIBRAS (Invitrogen), 200 µL volumes for well; 37°C, 5% CO2.

### Gyros Assay

1. At days 0-2, mouse compound solutions were incubated in HDK concentration (HD): 100 µL per well. Kinase inhibitor: CalphaChex-10 µM (PKC); inhibitor: CalphaChex-10 µM (PKC); staurosporine (10 µM) was added to the solution 15 minutes prior to the HDK concentration.

### Western Blot

1. After primary incubation, bands were washed 3 times in PBS with 0.1% Tween-20; 2. Blots were blocked in 5% nonfat dry milk for 1 hour prior to blotting.

### GSK3β Inhibition

The GSK3β inhibitor PF-04820367 did not decrease phosphorylation at the AT8 or pSer262 sites.

### Tau Suppression in a Neurodegenerative Mouse Model Improves Memory Function

1. The JNK pathway amplifies and drives subcellular changes in tau phosphorylation.

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