Journal of Alzheimer's Disease 22 (2010) 1–4
IOS Press

Supplemental Data

The K_{ATP} Channel Activator Diazoxide Ameliorates Amyloid-β and Tau Pathologies and Improves Memory in the 3xTgAD Mouse Model of Alzheimer's Disease

Dong Liu[a,1], Michael Pitta[a,1], Jong-Hwan Lee[a,b], Balmiki Ray[c], Debomoy Lahiri[c], Katsutoshi Furukawa[a], Mohamed Mughal[a], Haiyang Jiang[a], Julissa Villarreal[d], Roy G. Cutler[a], Nigel H. Greig[a] and Mark P. Mattson[a,e,*]

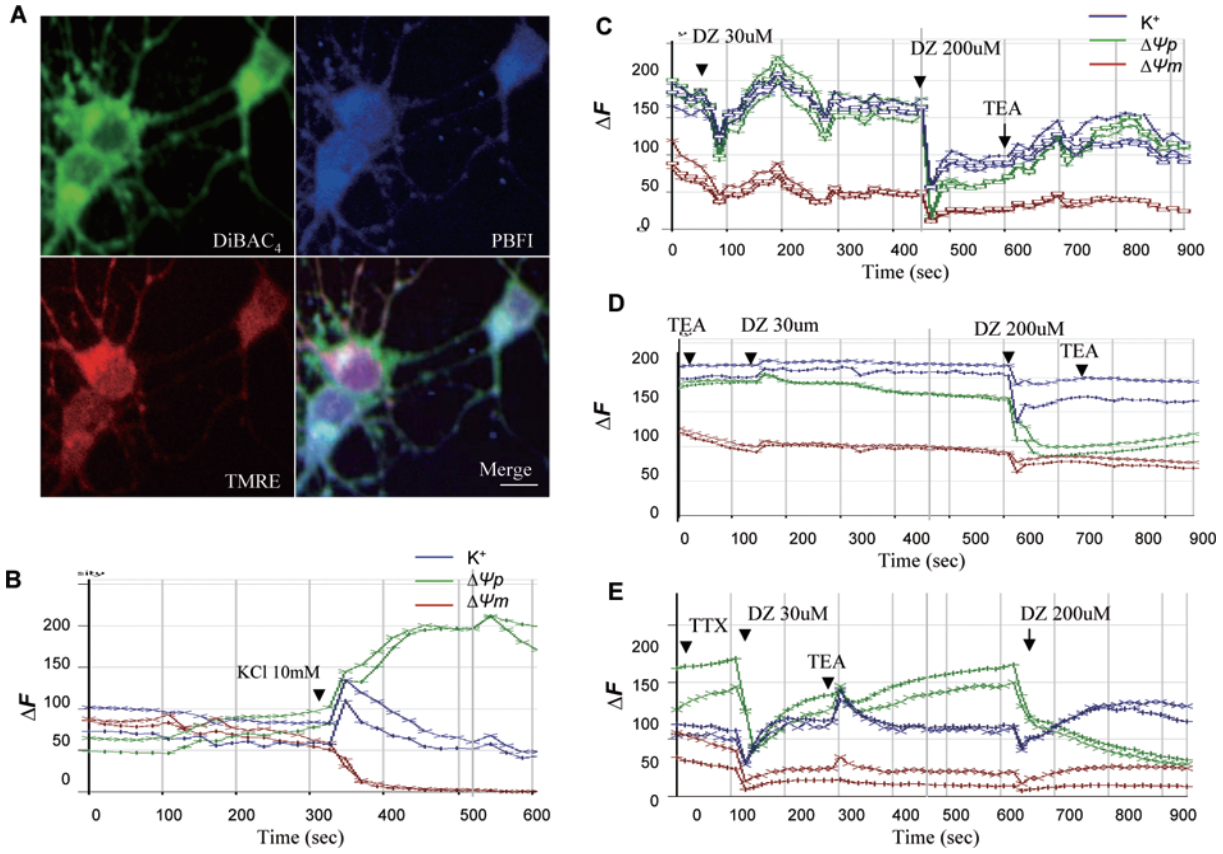
[a]*Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, Baltimore, MD, USA*
[b]*Department of Anatomy, College of Veterinary Medicine, Konkuk University, Seoul, South Korea*
[c]*Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN, USA*
[d]*Laboratory of Experimental Gerontology, National Institute on Aging Intramural Research Program, Baltimore, MD, USA*
[e]*Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Accepted 27 June 2010

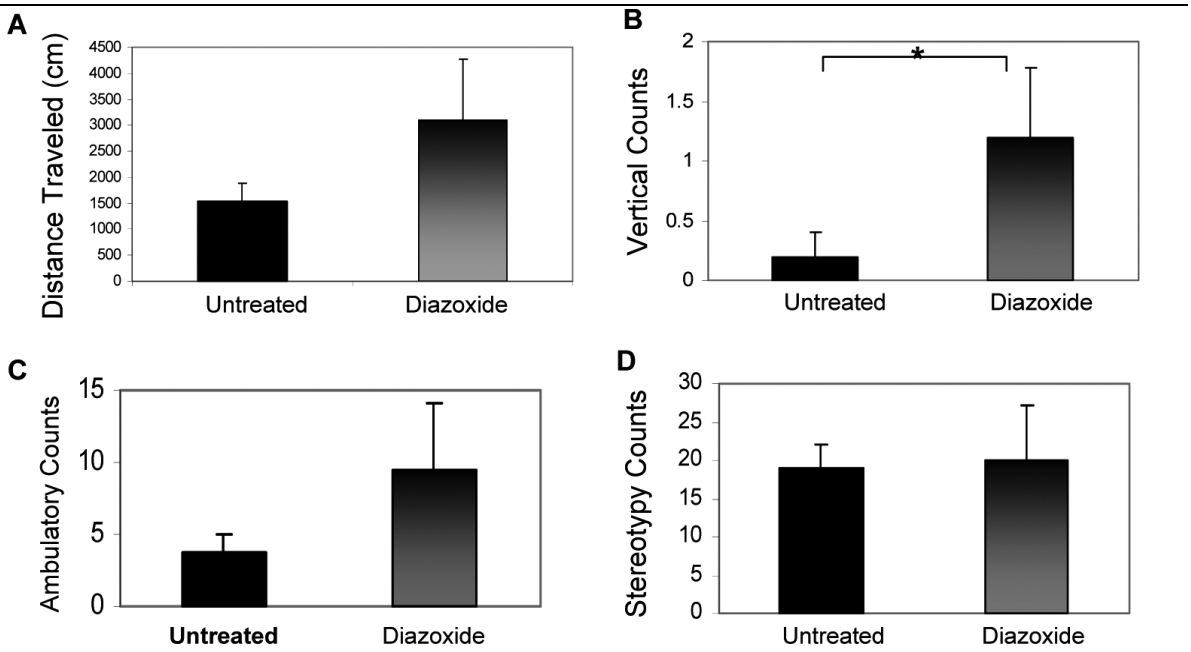
[1]These authors contributed equally.

*Correspondence to: Mark P. Mattson, National Institute on Aging Intramural Research Program, Biomedical Research Center, 251 Bayview Boulevard, Baltimore, Maryland 21224, USA. E-mail: mattsonm@grc.nia.nih.gov.

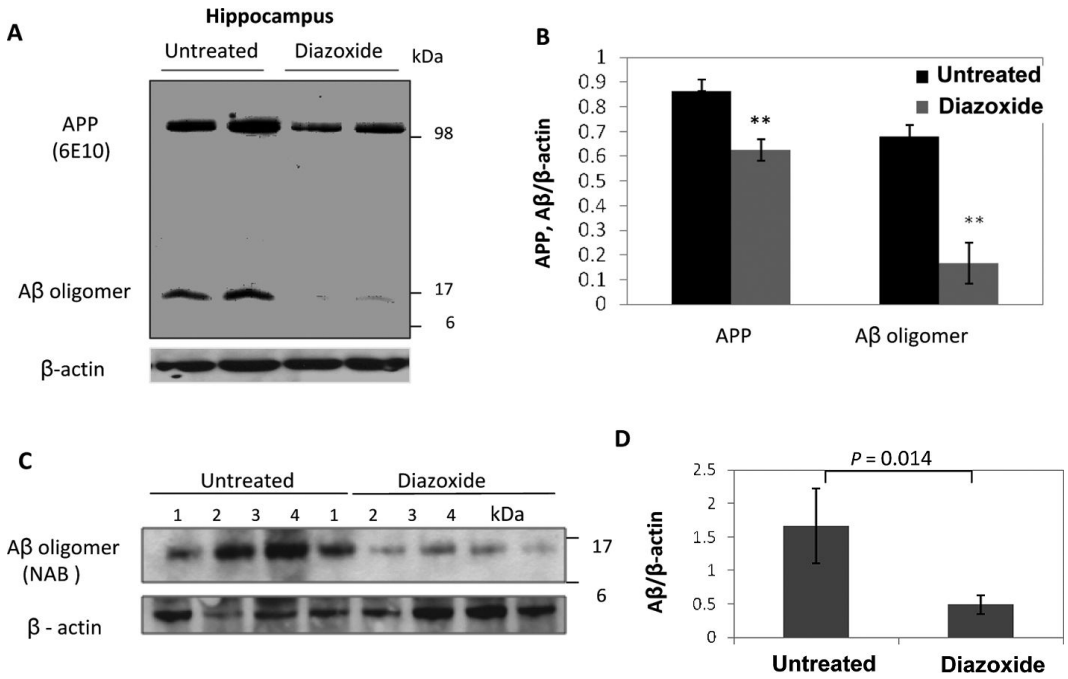
ISSN 1387-2877/10/$27.50



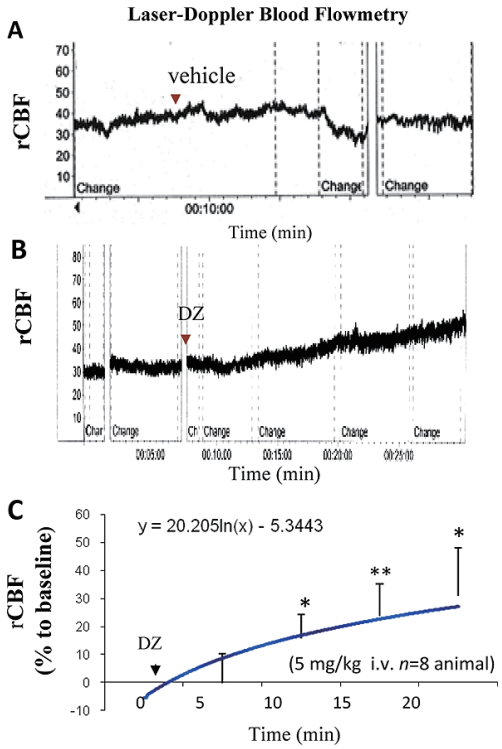
Supplemental Figure 1. Diazoxide hyperpolarizes the plasma membrane and reduces intracellular K^+ levels in a Na^+ channel-independent manner. A) Representative images of neurons loaded with $DiBAC_4$ a probe for $\Delta\Psi p$ (green), TMRE a probe for $\Delta\Psi m$ (red), and PBFI a probe for intracellular K^+ levels (blue). B) The intracellular K^+ concentration, $\Delta\Psi p$ and $\Delta\Psi m$ were measured prior to and during exposure of neurons to 10 mM KCl to depolarize the plasma membrane. C-E) The intracellular K^+ concentration, $\Delta\Psi p$ and $\Delta\Psi m$ were measured prior to and during exposures of neurons to the indicated treatments. Images were acquired sequentially at Ex and Em wavelengths for each probe every 10 s and each trace represents the average pixel intensity (ΔF) recorded from the cell body of an individual neuron. DZ, diazoxide; TEA, tetraethylammonium (1 mM); TTX, tetrodotoxin (500 nM). Each trace is the recording from an individual neuron. Similar results were obtained in at least three separate experiments.



Supplemental Figure 2. Evidence that diazoxide treatment reduces anxiety-like behavior in 3xTgAD mice. Results of measurements of four different variables in the open field test: A) Distance traveled; B) vertical movements (rearing on hind limbs); C) Ambulatory counts; and D) Stereotyped movements (licking and grooming). Compared to control 3xTgAD mice the diazoxide-treated 3xTgAD mice exhibited significantly more vertical counts and trends towards increased ambulatory activity and distance traveled, suggesting lower levels of anxiety in the diazoxide-treated mice.



Supplemental Figure 3. Diazoxide treatment reduces levels of holo-AβPP and Aβ oligomers in the hippocampus of 3xTgAD mice. A) Immunoblot using Aβ/AβPP antibody 6E10 showing levels of holo-AβPP and Aβ oligomers in samples of hippocampal tissue from control and diazoxide-treated 3xTgAD mice. B) Immunoblot (upper) and results of densitometric analysis (graph) of Aβ oligomers in samples from the hippocampi of control and diazoxide-treated 3xTgAD mice.



Supplemental Figure 4. Diazoxide increases cerebral blood flow. A, B) Representative recording of cerebral blood flow (CBF) prior to and after intravenous administration of vehicle (saline) and diazoxide (5 mg/kg) in mice. C) Summary data of CBF recordings following diazoxide administration showing that diazoxide administration rapidly increases CBF. Values are the mean and SD ($n = 8$ mice). $*p < 0.05$, $**p < 0.01$ compared to the baseline value.