Hypoglycemia causes widespread white matter injury - an imaging study in transgenic mice

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INTRODUCTION

Insulin-induced hypoglycemia presents the most important limitation to effective treatment for diabetes. Acute severe hypoglycemia may cause transient or permanent brain dysfunction such as confusion, cognitive impairment, seizures and coma. Deprivation of cellular glucose also contributes to the pathophysiology of ischemic brain injury. Recent neuroimaging and pathological studies of patients with severe hypoglycemic episodes suggest that white matter is also vulnerable to hypoglycemia. Although hypoglycemic brain injury is well documented in gray matter, little is known of mechanisms of injury in white matter deprived of glucose. In this study, we investigated the time course *in vivo* of axon and oligodendrocyte injury in a model of cerebral white matter injury in acute brain slices from adult transgenic mice. Brain slices permit direct access to white matter cellular components within an intact 3-D relationship and are therefore ideal to examine white matter injury without regard to alterations in cerebral vasculature or blood flow, which are known to be effected by diabetes. YFP labeling of axons in white matter proved as a very sensitive marker for visualization of axonal injury *in vivo*. These results indicate that glucose deprivation causes delayed structural disruption in axons, mediated in part by activation of AMPA/kainate glutamate receptors. Transgenic expression of fluorescent proteins allows direct observation of cell-specific structural changes in living tissue.

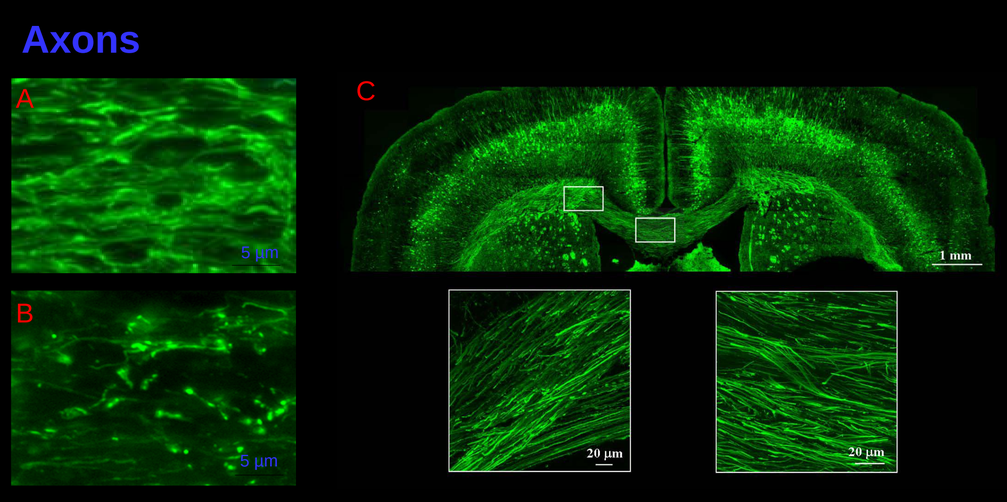

Fig 2. Thy1-YFP mouse allows visualization of detailed axonal structure.

A: Immunofluoresence micrograph showing SMI-31 labelling of axons in control slice of corpus callosum (c.c). Note linear fibers and bright fluorescence. B: brain slice exposed to 45 min of GD. Note the formation of axonal heads and retraction bulbs and the loss in fluorescence reminiscent of irreversible axonal injury and structural breakdown. C: Montage showing the intense expression and distribution of YFP in cortical layer 5 with axonal projections across c.c and striatum exploiting fluorescence imaging. Below: Maximum Intensity Projections from 16µm fixed sections showing how axonal tracts project along and across the c.c.

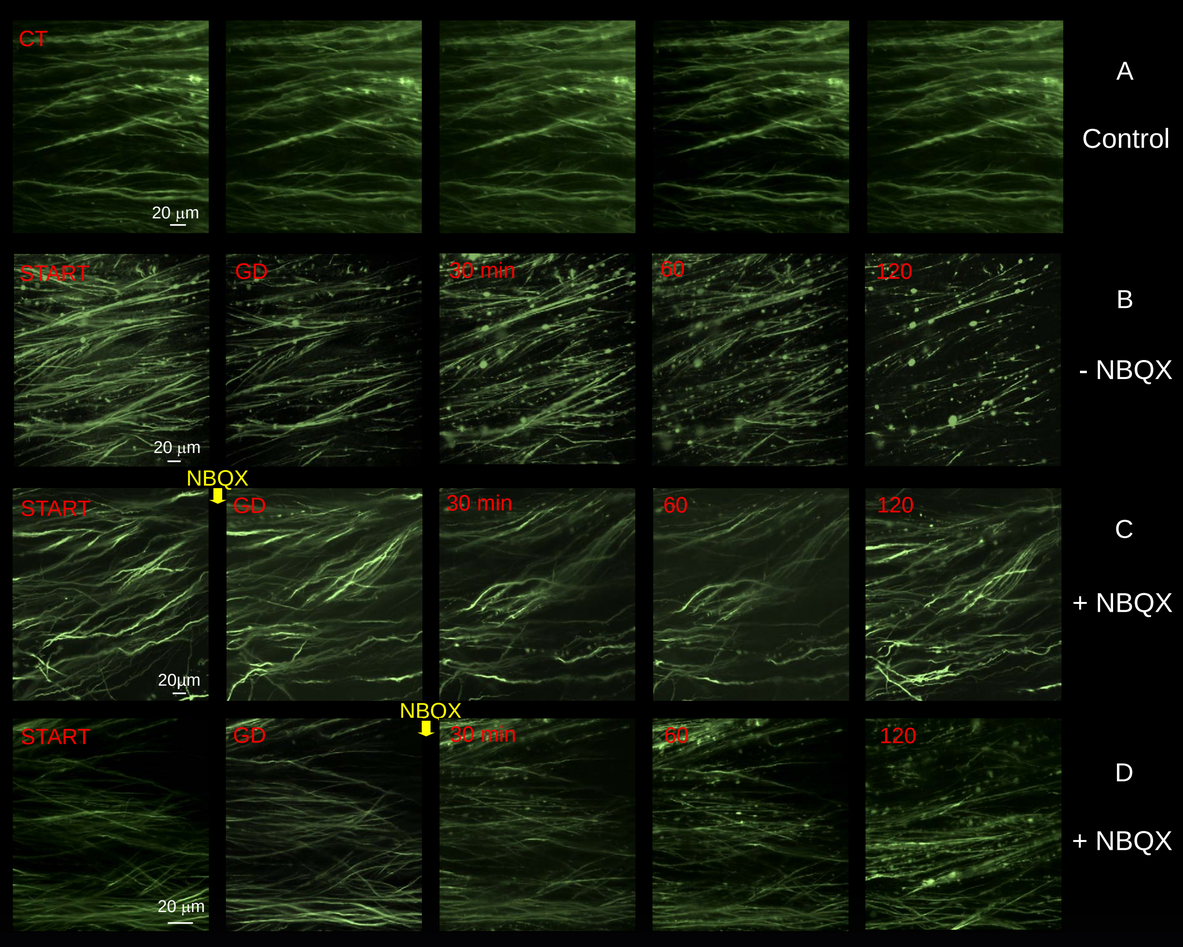

Fig 4. Transient glucose deprivation (GD) causes delayed axonal injury in adult mouse brain slices.

AMPA/kainate receptor blockade administered during or after GD substantially preserves axonal structure. Confocal laser-scanning microscopy permitted high resolution sequential time-lapse imaging deep in white matter with spatial and temporal resolution sufficient to follow morphological changes in axons. Each image is a maximum intensity projection of 20 optical sections spaced 1µm apart, and extending to a depth of 90µm below the slice surface.

Row (a) Brain slice remained structurally intact as long as four hours at ~ 33°C.
Row (b) Slice exposed to 45 min of transient GD showed axonal injury which develops starting 30 min after restoration of glucose. Extensive beading and fragmentation of axons leads to severe axon loss as shown by the progressive loss in fluorescence.
Row (c) Slice exposed to 45 min of GD with NBQX applied 30 min prior, during and after the insult shows well preserved axons. (d) Slice exposed to 45 min of GD with NBQX applied after the insult shows good axonal preservation. Beading and fragmentation of axons is less pronounced and a proportion of axons generally maintain their integrity.

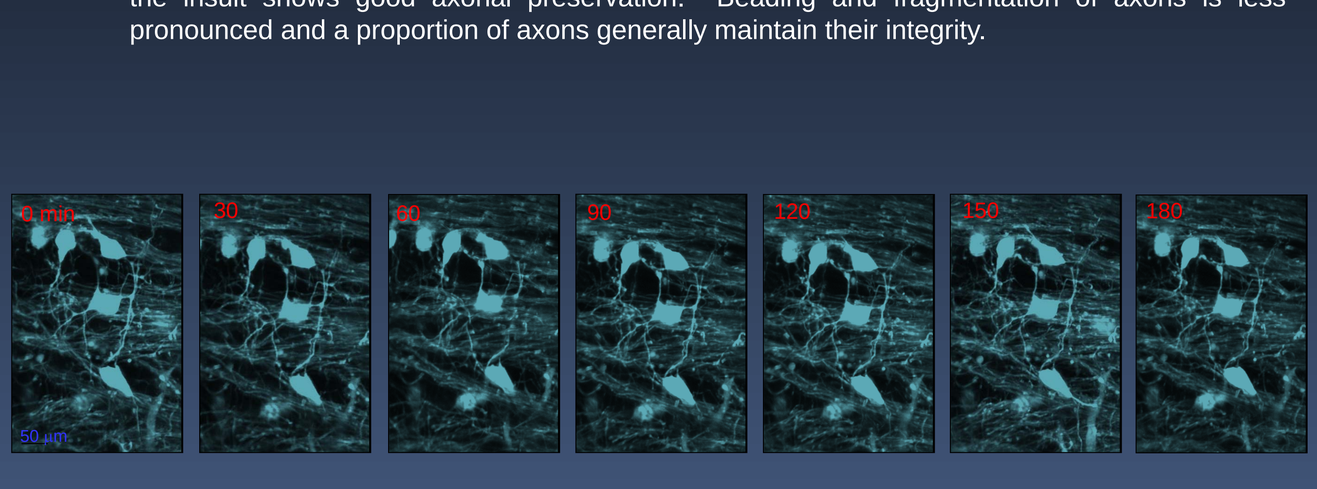

Fig 6. Microscopy allowed the sequential imaging of PLP-eGFP oligodendrocytes from live brain slices at high spatial resolution within their native environment. Sequential imaging of acutely isolated brain slice (400 µm) under control conditions shows fine detail of the oligodendrocyte cell bodies and processes in corpus callosum over three hours.

References

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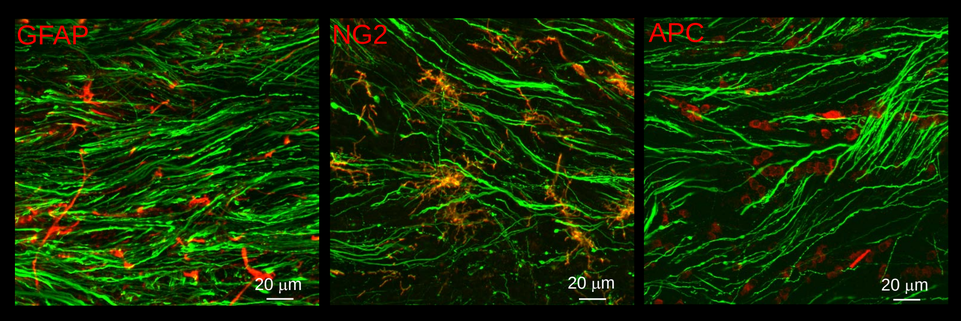

Fig 3. Visualization of axon-glia interactions in the corpus callosum.

Confocal micrographs in control fixed tissue show the relationship of YFP-labeled axons to astrocytes (anti-GFAP), oligodendrocyte progenitor cells (anti-NG2), and oligodendrocytes (anti-APC, clone cc-1).

METHODS

Acute brain slice preparation: After deep halothane anesthesia and decapitation, the cranium was opened and the brain rapidly removed and placed in ice-cold and oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid buffer (aCSF) supplemented with 75mM sucrose. aCSF composed of (in mM) 126 NaCl, 3.5 KCl, 1.3 $MgCl_2$, 2.0 $CaCl_2$ 1.2 NaH_2PO4, 25 $NaHCO_3$, and 10.0 glucose, pH 7.4. Sucrose containing physiological saline was composed of 87 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO4, 0.5 mM $CaCl_2$, 3 mM $MgCl_2$, 25 mM glucose, 75 mM sucrose, pH 7.3. After removing the cerebellum and brainstem, the entire brain was mounted on the platform of a Vibratome 1500 vibroslicer, covered in the appropriate buffer (ice-cold and oxygenated) and 400µm thick sections were cut in the coronal plane. Only the slices in which the anatomical structure of the corpus callosum was clearly visualized was included in the experiments. Immediately after sectioning, slices were allowed to recover at room temperature in oxygenated buffer for 2 h in a Haas-type slice chamber (Harvard Instruments) at a flow rate of 4.0 ml/min before they were transferred for imaging.

Imaging live brain slice with confocal microscopy: A mini submerged chamber (0.5ml) with a coverglass bottom (Warner Instrument Corporation, Hamden, CT) was mounted on an inverted laser scanning confocal microscope system. Slices were placed in the submerged chamber and superfused with oxygenated aCSF at a flow rate of 6.0 ml/min.

Final temperature control (33 ± 1°C) was maintained using an in-line heater (Warner Instruments) equipped with a feedback thermistor placed in the chamber and raised gradually over 1 h. Perfusion was performed by gravity flow into the chamber and vacuum aspiration within the chamber. A gravity flow regulator in the perfusion line controlled the flow rate. YFP labeled axons were imaged with a 488 nm laser line using a 40X water immersion lens through the bottom of the perfusion chamber. For EGFP, imaging was performed at 488nm using a 63X water immersion lens. Twenty z-series optical sections were collected at an incremental z-step of 0.5µm apart and collapsed to give a maximum intensity projection of each sequential time series. Confocal laser-scanning microscopy permitted high resolution sequential time-lapse fluorescence imaging deep in white matter slices (up to 50µm) with minimal photodamage. Cutting damage typically extended approximately 20–30µm from the slice surface. Using confocal laser scanning microscopy, we were able to image healthy appearing YFP labeled axons restricted to the first 40-50 µm deep were preparation damage was minimal. However, as expected, the fluorescence signal dropped off substantially at greater depths. Glucose deprivation (GD) was initiated by replacing glucose in normal aCSF with 10mM sucrose. For drug application, the oxygenated buffer was replaced with aCSF supplemented with 30 µM NBQX.

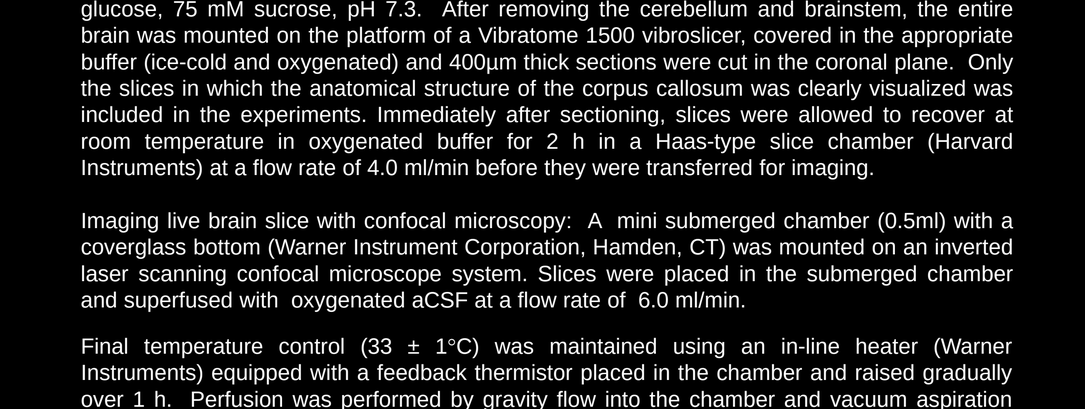

Fig 5. Transgenic mice with oligodendrocyte expression of fluorescent proteins under the proteolipid protein (PLP) promoter. Confocal micrographs show white matter of PLP-eGFP (W. Macklin Lab) and PLP-DsRED (F. Kirchhoff Lab) transgenic mice in control fixed sections (16µm) at low and high magnification. Fluorescence was brighter in mice expressing eGFP than dsRed.

CONCLUSIONS

- White matter in brain slices from transgenic fluorescent mice remained structurally intact as long as four hours at ~ 33°C, sufficient to assess slowly developing processes such as axonal structural disintegration and oligodendrocyte morphology.

- Our acute brain slice model using YFP-H transgenic mice is a reliable model to follow axonal injury following GD, as it allows a detailed sequential assessment of axon morphology, suitable for multiphoton imaging without the reliance on traditional cytoskeleton labelling techniques normally employed in fixed tissue preparations.

- In this model, transient glucose deprivation (GD) caused delayed axonal injury, developing 30-120 min after restoration of glucose. NBQX, substantially preserved axon structure even if given after completion of GD.

- In the transgenic PLP-EGFP and PLP-dsRED mice, oligodendrocytes could easily be detected in white and gray matter due to their high level of expression. The detailed morphology of PLP-EGFP expressing oligodendrocytes could be visualized at a higher resolution in 16µm-thick brain sections and in living slices (400µm).