

## Vaccination with myelin-antigens exacerbates loss of axotomized facial motor neurons in mice

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Within the CNS, T-lymphocytes accumulate near the soma of axotomized peripheral nerves. Using a model of facial nerve transection (FNTx), a number of studies have established a role for endogenous T-cell-mediated neuroprotection of axotomized facial motoneurons (FMN). Although the antigen specificity of T-cell mediated neuroprotection has not been defined after FNTx, other reports suggest that myelin-reactive T-cells can provide protection to injured CNS neurons. To determine if myelin-reactive T-cells influence survival of axotomized FMN, we vaccinated adult C57BL/6 mice (n=78) with myelin basic protein (MBP) or myelin oligodendrocyte glycoprotein (MOG) 7 or 21d prior to FNTx. These intervals were chosen to exploit the peak effector functions of acutely activated lymphocytes (7d; acute) or memory T-cells that might be reactivated by nerve injury (21d; delayed). Control groups consisted of FNTx mice without immunization or FNTx mice immunized with ovalbumin (OVA; a non-CNS protein). All antigens were emulsified in complete Freund's adjuvant then were injected intradermally. Equidistant tissue sections cut through the brainstem and encompassing the facial nucleus were prepared for all mice 14 or 28 days after FNTx. Bilateral FMN counts were obtained from cresyl violet-stained tissue sections (0.02 mm thickness) using optical fractionators—a stereological counting method that yields unbiased estimates of total neuron numbers. Briefly, uniform, random sampling was used to identify a percentage (~ 6%) of total tissue for analysis. Within that sample, only FMNs having a well-defined nucleus/nucleolus were tallied and FMN counts were determined by dividing number of tallied FMN by the sampling fraction (~ 6%). Data are reported as % FMN loss relative to the contralateral (uninjured) side. After acute immunization, MBP-vaccination exacerbated FMN loss compared to OVA-immunization at 14d (MBP: 49% loss, OVA: 23% loss,  $p<0.01$ ) and 28d survival times (MBP: 74% loss, OVA: 61% loss,  $p<0.05$ ). Similarly, MBP-vaccination 21d prior to injury augmented FMN loss at 14d (MBP: 37% loss, OVA: 12% loss,  $p<0.01$ ) and 28d survival times (MBP: 61% loss, OVA: 42% loss,  $p<0.01$ ). Interestingly, MBP T-cell receptor transgenic mice subjected to FNTx (mice with >95% of their endogenous CD4+ T-cell repertoire specific for MBP; n=16), failed to show an exacerbation of FMN loss, compared to non-transgenic littermates (n=16). Increased neuron loss was seen only at 28d in delayed-MOG-immunized mice (57% loss,  $p<0.05$  vs. OVA). Collectively, these data indicate that *in vivo* expansion of myelin-reactive T-cells via vaccination exacerbates FMN loss after axotomy. However, peripheral nerve injury alone is insufficient to cause biologically significant endogenous T-cell activation. These data have important implications for vaccine therapies designed to augment autoimmune responses in clinical neurotrauma. Supported by NIH T32 AI55411 (DPA) & NS740218 (PGP).

## **Zebrafish *topped* is Required for Ventral Motor Axon Guidance**

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Zebrafish primary motor axons extend along stereotyped pathways innervating distinct regions of the developing myotome. During development these axons make stereotyped projections to ventral and dorsal myotome regions. Caudal primary motoneurons, CaPs, pioneer axon outgrowth along ventral myotomes, whereas, middle primary motoneurons, MiPs, extend axons along dorsal myotomes. Although the development and axon outgrowth of these motoneurons has been characterized, cues that determine whether axons will grow dorsally or ventrally have not been identified. The *topped* mutant was previously isolated in a genetic screen designed to uncover mutations that disrupt primary motor axon guidance. CaP axons in *topped* mutants fail to enter the ventral myotome at the proper time, stalling at the nascent horizontal myoseptum, which demarcates dorsal from ventral axial muscle. Later developing secondary motor nerves are also delayed in entering the ventral myotome whereas all other axons examined, including dorsally projecting MiP motor axons, are unaffected in *topped* mutants. Genetic mosaic analysis indicates that Topped function is non-cell autonomous for motoneurons and when wild-type cells are transplanted into *topped* mutant embryos, ventromedial fast muscle are the only cell type able to rescue the CaP axon defect. These data suggest that Topped functions in the ventromedial fast muscle and is essential for motor axon outgrowth into the ventral myotome.

## The 'Stop and Go' Model of Slow Axonal Transport: Experiments *In Silico*

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Radio-isotopic pulse-labeling studies in laboratory animals have shown that neurofilaments are transported along axons in Slow Component 'a' of axonal transport, at average rates of 0.002-0.035 $\mu\text{m/s}$  (0.2-3mm/day). In contrast, direct observations in cultured nerve cells using time-lapse fluorescence imaging have demonstrated that neurofilaments actually move much faster, at average rates of 0.4-0.7 $\mu\text{m/s}$ . However, they also move in both anterograde and retrograde directions and they spend most of their time not moving. These observations suggest that slow axonal transport is generated by rapid bidirectional movements interrupted by prolonged pauses. To test whether this 'stop and go' hypothesis can explain the kinetics of slow axonal transport *in vivo*, we have developed a stochastic model for the axonal transport of neurofilaments based on our time-lapse imaging data. We find that rapid, intermittent, bidirectional and asynchronous movement of axonal neurofilaments can match the kinetics of neurofilament protein transport obtained by radio-isotopic pulse-labeling in mouse sciatic nerve motor axons. Our modeling suggests that: (1) on average, neurofilaments in mouse sciatic nerve motor axons spend 97% of the time pausing during their journey towards the axon tip, (2) pauses are prolonged (average=430 seconds), (3) reversals occur but they are rare (average=0.09/filament/hour), (4) the frequency and duration of the pauses and reversals are likely to be the principal determinants of the net transport rate and steady state distribution of neurofilaments in axons. We conclude that the motile behavior of individual neurofilaments observed in cultured neurons can account for the population kinetics of slow axonal transport observed *in vivo*.

## Ribosomal S6 Kinases couple the MAPK cascade to expression of the core clock gene mPer1

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The mitogen activated protein kinase (MAPK) cascade has been shown to couple photic stimulation to entrainment of the mammalian circadian clock. MAPK signaling regulates transcription via the direct actions of extracellular signal regulated kinase (ERK) 1/2 on transcription factors and indirectly through ERK-regulated kinases. One such downstream target of ERK is the family of 90 kDa ribosomal S6 kinases (RSKs). RSKs stimulate gene transactivation via the regulation of both DNA binding proteins, including c-Fos and CREB, and cofactors such as CBP and p300. Photic stimulation has been shown to trigger MAPK-dependent activation of RSK1 during the subjective night, suggesting that RSK1 may contribute to MAPK-dependent entrainment of the circadian clock. To determine whether ERK and RSKs regulate clock gene expression, we employed an *in vitro* luciferase reporter gene system. Rat-1 cells were transfected with constitutively active or dominant negative RSK and MAPK constructs and the effects on mPer1- and mPer2-reporter gene expression were examined. We observed that both RSK and MAPK signaling significantly stimulated mPer1 expression, but that mPer2 expression was not altered. RSKs coupled to mPer1 via CREB-dependent and -independent signaling events. Further studies will be performed to examine the specific mechanisms by which ERK and RSKs stimulate clock gene transcription. These data provide new insights into the cellular signaling events that regulate mPer1 expression. Supported by grants from the National Science Foundation (IBN-0090974) and the National Institute of Mental Health (MH-62335).

## Characterization of a Mild Excitotoxic Injury in the Rat Spinal Cord White Matter

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After a traumatic spinal cord injury (SCI), the spinal cord fails to effect complete repair. Instead, substantial secondary injury results in larger, more debilitating lesions. One likely mechanism of this secondary injury is excitotoxicity, mediated through glutamate and tumor necrosis factor alpha (TNF $\alpha$ ). It was recently shown that TNF $\alpha$  can potentiate the excitotoxic effects of glutamate, possibly through upregulation of glutamate receptors. Both TNF $\alpha$  and glutamate are released in high concentrations after SCI, where they can elicit these toxic effects on cells around the primary lesion. Here we investigate excitotoxic pathways, which may mediate secondary injury in the spinal cord white matter, using a microinjection model.

We injected kainate (KA, a glutamate agonist) alone, TNF $\alpha$  alone, or kainate plus TNF $\alpha$  into the exposed T10 spinal cord after laminectomy. Animals were sacrificed at various time-points after injection and cords were examined for morphological and immunohistological signs of tissue damage. Ninety minutes after gray matter injection, gross tissue damage and neuronal cell loss were evident (Hermann et al., 2001, *Neurobiol. Dis.* 8:590-9). At the same time-point, no damage or cell loss was seen after white matter injection. However, by 2 days after injection into the white matter, large lesions were evident, with major disruption of axons and oligodendrocytes. White matter lesions extended rostrocaudal to the injection site, but not laterally. Control animals, injected with albumin, showed that the injection procedure alone caused no damage. Although previous studies have shown the effects of excitotoxicity in the gray matter, we show here that there is also a delayed excitotoxic effect in the white matter. Our characterization of this delayed white matter damage emphasizes the likely role of excitotoxicity in secondary damage after SCI, and suggests that preventing this excitotoxicity is a viable strategy for therapeutic intervention.

**ANATOMICAL EVALUATION OF DAMAGE PRODUCED BY GRADED UNILATERAL CERVICAL SPINAL CORD CONTUSION INJURY IN RATS** J.C.Gensel<sup>1\*</sup>; C.Tovar<sup>1</sup>; F.P.T.Hamers<sup>2</sup>; M.S.Beattie<sup>1</sup>; J.C.Bresnahan<sup>1</sup>

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Contusive human spinal cord injury (SCI) is a chronic, debilitating condition with highest incidence in young adults. Cervical SCI is the most common and devastating; leaving patients paralyzed with a 15-20 year decreased life expectancy. Most animal SCI contusion models focus on thoracic injury to examine regeneration, repair, and sprouting of long, white matter tracts. The current study characterizes the anatomical damage produced by unilateral cervical contusions in the rat. The relationship between this damage and the biomechanical descriptors of the injury and functional outcome measures were also evaluated. Anatomical measurements included: sparing at lesion epicenter; sparing sampled throughout the rostral-caudal extent of lesion; the intensity of myelin staining in specific areas of the cord rostral and caudal to the lesion epicenter; 3-D reconstructions of the lesion; and quantification of motor neuron loss. Sham surgeries, mild (6.25mm), and moderate (12.5mm) unilateral SCI (n=10-11 per group) were made at C5 with the NYU/MASCIS weight-drop device. All measures were evaluated 6-weeks post-SCI. Sparing measures revealed SCI severity-specific differences in white and gray matter sparing at lesion epicenter (mean % total spared area of ipsilateral hemi-cord vs. the contralateral hemi-cord: 6.25mm=36.0%, 12.5mm=17.7%). Examination of sparing through the rostral-caudal extent of the lesion revealed similar results (mean % of spared area: 6.25mm=67.7%, 12.5mm=48.3%). Less myelin staining was detected in ventral/lateral funiculi caudal to the lesion epicenter in the 12.5mm vs. 6.25mm group. 3-D reconstructions revealed elliptical shaped lesions in both SCI groups. There was a significant loss of motor neurons in the hemi-cord ipsilateral vs. contralateral to the injury, however this differences was not significant between SCI groups. Histological measures correlated to behavioral tests and biomechanical descriptors of the injury. The extensive characterization of this model makes it a promising tool for studying SCI.

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## **LESION DEVELOPMENT AND NEUROINFLAMMATION IN SPINAL INJURED MICE: CORRELATIONS WITH GENETIC SUSCEPTIBILITY TO CNS EXCITOTOXICITY AND AUTOIMMUNITY**

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Genetic differences in mice confer varying degrees of protection from autoimmune and neuroexcitotoxic pathology of the central nervous system (CNS). Here, we describe unique patterns of neurodegeneration and intraparenchymal inflammation following traumatic spinal cord injury (SCI) in mice that are resistant or susceptible to kainite excitotoxicity and experimental autoimmune encephalomyelitis (EAE). Within each strain (C57BL/6, C57BL/10, BALB/c and B10.PL), spinal cords were prepared for stereological analyses of lesion volume and for immunohistochemical characterization of inflammatory cell infiltrates at 6 hrs, 3, 14 or 42 days following mid-thoracic (T9) spinal contusion injury. The data revealed no relationship between the extent of secondary neurodegeneration and susceptibility to EAE or excitotoxicity. Namely, in excitotoxic-resistant mice (C57BL/6 and Balb/c), we noted the largest lesion volumes and longer axial spread of neurodegeneration compared to C57BL/10 and B10.PL mice. End-stage analysis of locomotor function correlated with morphometric indices of lesion volume/length. In contrast, the magnitude of intraparenchymal inflammation correlated with susceptibility to EAE but not morphometric or locomotor outcome. Specifically, in C57BL/6, B10.PL, and C57BL/10 mice, maximal macrophage infiltration was noted at the injury site between 3 and 14 dpi with a subsequent decline in the intensity of labeling between 14 and 42 dpi. Acute macrophage influx was similar in Balb/c mice, however, significantly less macrophage activation was evident at 14 and 42 dpi. Lymphocytic infiltrates were absent from all strains until 14 dpi, after which cell numbers increased steadily within the lesion site through 42dpi. Despite the similar kinetics of lymphocyte infiltration, significantly fewer T-cells infiltrate the spinal cord of Balb/c mice at all post-injury times examined. Expression of class II MHC molecules also was present in all strains and remained elevated from 14 to 42 dpi. However, class II molecules were elevated to the greatest extent in C57BL/6 mice. These data reveal strain-dependent neuroinflammatory responses following SCI in mice. Interestingly, despite the designation of these strains as EAE- or excitotoxic-resistant/susceptible, the magnitude and composition of the inflammatory response triggered by SCI did not have predictive value for interpreting lesion morphometrics or behavioral outcome.

## Proteomic Analysis of the Brains from Mice that Lack a Growth Hormone Receptor

Doug Kohn

Growth hormone (GH) regulates cell growth and differentiation primarily by modulating gene expression and metabolism in target tissues. Targeted disruption of the gene encoding the growth hormone receptor and binding protein (GHR/BP<sup>-/-</sup>) functionally inactivates GH and generates long-lived, dwarf mice with elevated circulating GH and markedly reduced insulin-like growth factor-1 (IGF-1) levels (1, 2). Indeed, insulin/IGF-1 signaling has been shown to be a critical determinant of lifespan in several species. GHR/BP<sup>-/-</sup> mice also have decreased fasting insulin and glucose levels (3) and appear to resist complications due to streptozotocin (STZ)-induced diabetes (4). To determine the consequences of the GHR/BP<sup>-/-</sup> mutation on gene expression in the central nervous system (CNS), brain tissue was harvested from normal and gene-disrupted mice at different developmental stages (neonate, adult and aged) and proteins were isolated from distinct subcellular fractions (nucleus, cytoplasm, polysomes) using differential gradient ultracentrifugation. The proteins in each fraction were resolved by two-dimensional gel electrophoresis and stained with the fluorescent dye SYPRO Orange. The images were captured with a high-resolution CCD camera (Bio-Rad Versa-Doc 3000) or a laser-scanning device (Fuji FLA-3000G) and quantitatively analyzed with PDQuest or Image Gauge software packages. Differentially expressed proteins were manually excised from the gels and identified by mass spectrometry. Of the hundreds of proteins resolved, several were differentially expressed in the brains of GHR/BP<sup>-/-</sup> mice relative to controls. The goal is to identify those proteins whose expression patterns are spatially and temporally correlated and establish functional protein networks that may delay or attenuate age-related tissue dysfunction or diabetic complications. *This work was supported in part by the State of Ohio's Eminent Scholar Program, which includes a gift from Milton and Lawrence Goll.*

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## ACTIVITY-DEPENDENT NEUROPROTECTION AND CREB: KINASE COUPLING, STIMULUS INTENSITY, AND TEMPORAL REGULATION OF CREB PHOSPHORYLATION.

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The dual nature of the NMDA receptor as a mediator of excitotoxic cell death and activity-dependent cell survival likely results from divergent patterns of kinase activation, transcription factor activation and gene expression. To begin to address this divergence, we examined cellular and molecular signaling events that couple toxic and non-toxic levels of NMDA receptor stimulation to activation of the CREB/CRE pathway in cultured cortical neurons. NMDA receptor stimulation elicited by a brief (10 min) pulse of synaptic activity triggered a sustained increase (up to 3 hrs) in the Ser-133 phosphorylated form of CREB (pCREB) and activation of CRE-mediated transcription. In contrast, brief stimulation with a toxic concentration of NMDA (50  $\mu$ M) triggered a transient (25-45 min) form of pCREB that did not stimulate transcription. pCREB duration was determined by calcineurin. Fifty  $\mu$ M NMDA potently stimulated calcineurin activity and the inhibition of calcineurin reduced NMDA toxicity and converted the transient increase in pCREB into a sustained increase. Signaling via the ERK/MAPK and CaMK pathways was required for the induction but did not determine the duration of synaptically-evoked CREB phosphorylation. Fifty  $\mu$ M NMDA attenuated the duration of synaptically-evoked pCREB, but only if it was applied after the kinase-dependent induction phase. Constitutively active and dominant-negative CREB constructs were used to implicate CREB in synaptic activity dependent neuroprotection against NMDA-induced toxicity. Together these data provide a framework to begin to understand how the neuroprotective and toxic effects of NMDA receptor activity function in an antagonistic manner at the level of the CREB/CRE transcriptional pathway.

## **Free 3-nitrotyrosine Potentiates Dopamine Mediated Neuronal Cell Death**

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The mechanisms responsible for neuronal cell death in Parkinson's disease (PD) remain largely unknown. Evidence suggests that cellular oxidative events may be key mediators of dopaminergic neuron loss. Protein nitration by peroxynitrite has long been an indicator of oxidative stress and it may also affect protein function. Similarly, nitration of free amino acids, namely free 3-nitrotyrosine (free-3NT), may alter their normal biological role. Previous studies demonstrated that intrastriatal injections of free-3NT in mice yielded specific loss of tyrosine hydroxylase immunoreactivity in both the striatum and substantia nigra, indicating loss of dopaminergic neurons. Additionally, these animals exhibited physical symptoms similar to those in established mouse models of PD.

Dissociated mouse cortical neurons were treated with free-3NT and dopamine and assayed for cell viability, ATP content, oxidant production, and intracellular reduced glutathione. 24-hour treatment with free-3NT alone proved to be virtually non-toxic. In combination with exogenous dopamine, free-3NT greatly potentiated dopamine's known neuronal toxicity. Cells treated with both free-3NT and dopamine exhibited decreased ATP content, increased extracellular peroxide levels, and decreased cellular reduced glutathione than treatment with dopamine alone. These data suggest that free-3NT may sensitize neurons to oxidant toxicity, possibly mirroring the conditions that exist in Parkinsonian brains.

Photoperiod-Evoked Neuroplasticity: Short Days Reduce Spatial Learning and Memory Performance in Adult Male *Peromyscus leucopus*

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Because habitats outside of the tropics change dramatically on a seasonal basis, individuals have evolved season-specific adaptations to maximize fitness and survival. Day length (photoperiod) is an important cue to predict the time of year. In the lab, photoperiod alone triggers these physiological, morphological, and behavioral adaptations. For example, most rodents use day length to limit reproduction to the season when reproductive success and survival are maximized. Seasonally-breeding polygynous rodents have smaller spatial home ranges and decreased relative brain mass and hippocampus volume during the non-breeding season. A reduction in energy-expensive brain mass would convey considerable energetic savings during the winter, and we hypothesized that the decrease in hippocampal size reflects a deficit in hippocampal function. Specifically, we predicted that short, winter-like photoperiods would decrease hippocampal volume and spatial learning and memory performance in adult male white-footed mice (*Peromyscus leucopus*). To test this hypothesis, we maintained male white-footed mice in long or short days for 11 weeks, and tested spatial learning and memory performance using the Morris water maze. We are testing two mechanisms by which short days may affect the hippocampus separately or in concert: 1) dendritic complexity of hippocampal pyramidal cells decrease in short days, 2) Neurogenesis as measured by expression of neuron growth factor genes decreases in short days. After 7 and 8 training trials in the Morris water maze, short-day mice had longer latencies and took a less direct path to reach the hidden platform. These differences were not confounded by differences in swim speed. Brains of these mice were stained using the Golgi method and dendritic branching hierarchy, dendritic length, spine density, and hippocampal volume will be measured in 10 transverse sections of each brain. Gene expression of nerve growth factor, brain derived growth factor, basic fibroblast growth factor, and insulin-like growth factor will be compared in the hippocampus of individuals housed in long- or short days using quantitative PCR. Our results are the first to suggest that a seasonal pattern to cognitive function exists and is mediated by neuronal plasticity.

## **Arrival, reversal and departure of neurofilaments at the tips of growing axons**

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Time-lapse fluorescence imaging of GFP-tagged neurofilament proteins in cultured neurons has demonstrated that neurofilament polymers move along axons at peak rates considerably faster than the rate of axon growth. This suggests that anterogradely moving neurofilaments may “catch up” with the advancing growth cone. To test this hypothesis, we examined the kinetics of neurofilament movement in distal regions of growing axons using time-lapse fluorescence imaging. Filaments (average length=6.1 $\mu$ m; minimum=1.3 $\mu$ m, maximum=24.4 $\mu$ m) moved intermittently at rapid rates in both anterograde and retrograde directions (60% anterograde, 40% retrograde; n=300). The average retrograde velocity was significantly faster than the average anterograde velocity (0.53 and 0.34 $\mu$ m/s respectively,  $p < 0.001$ ), suggesting that these movements are generated by distinct motors. Most anterogradely moving filaments (80%) entered the growth cone, and most retrogradely moving filaments (81%) originated from the growth cone. A small number of filaments (9 out of 300) reversed direction while we were observing them. Six of these reversals occurred in the growth cone (average residency time=128s; minimum=4s, maximum=304s). These observations suggest that the majority of retrogradely moving filaments in the distal axon originated by reversal at the growth cone. Based on the relative frequency of arrivals and departures, we estimate that 67% of the filaments that entered the growth cone eventually reversed direction. The remaining 33% of the filaments, which did not reverse, were presumably recruited to form the neurofilament cytoskeleton of the newly formed axon. Our data suggest that neurofilament polymers are delivered rapidly and infrequently to growth cones and that local mechanisms in growth cones can cause these polymers to reverse their direction of movement. We speculate that these mechanisms may be regulated to control the net delivery of neurofilaments to the tips of growing axons.

## **Neurofilament proteins are transported along axons in the form of single polymers**

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Thin axons of cultured neurons often exhibit naturally occurring discontinuities in their axonal neurofilament array, resulting in short segments of axon that lack neurofilaments. Time-lapse fluorescence imaging of these gaps in neurons expressing GFP-tagged neurofilament protein has revealed the rapid infrequent movement of filamentous structures up to 39 $\mu$ m in length. We have proposed that these structures represent single neurofilament polymers. To test this hypothesis, we have developed a perfusion technique that enables us to stop moving filaments within the gaps. Filaments that moved into gaps were stopped by perfusing the cells with 0.02% saponin, which permeabilizes the axonal plasma membrane. The moving filaments stopped 4 to 36 seconds after starting the flow of perfusate (average=11 seconds, n=37), presumably due to the loss of soluble factors necessary for the motility. The captured filaments remained even after perfusion with 0.5% Triton X-100, indicating that they were not membranous. To test the hypothesis that the captured filaments were single neurofilament polymers, we visualized them by immunofluorescence microscopy using an antibody specific for NFL, which is present in all neurofilaments. Using quantitative digital image processing and analysis techniques, we compared the fluorescent intensity of the captured filaments with single and bundled filaments in detergent-splayed cytoskeletons of sister cultures that were otherwise processed in an identical manner. The mean normalized fluorescent intensity of paired neurofilaments (1.98, n=44) was double that of single neurofilaments (1.00, n=169) and this difference was significant ( $p < 0.05$ , unpaired t-test). The mean normalized fluorescent intensity of the captured neurofilaments (1.11, n=17) was not significantly different from that of single neurofilaments ( $p > 0.05$ , unpaired t-test), but it was significantly less than that of paired neurofilaments ( $p < 0.05$ , unpaired t-test). Using correlative light and electron microscopy, we confirmed that the moving structures are indeed single continuous neurofilament polymers. These data support our hypothesis that neurofilament proteins are transported along axons in the form of single polymers.

**JNK3 is required for p75-dependent death of neurons and oligodendrocytes after CNS trauma.** Tae Young Yune, Qi Ming Li, Anthony W. Harrington, Eun Joo Kim, Klaus Giehl, and Sung Ok Yoon. Center for Molecular Neurobiology and the Department of Molecular and Cellular Biochemistry, Ohio State University.

We have previously reported that p75 is the receptor that is responsible for inducing death of corticospinal neurons (CSN) following internal capsule lesion, and also oligodendrocytes after spinal cord injury. When cultured, oligodendrocytes undergo apoptosis upon p75 activation as they do in vivo, and activate JNK3, an injury-specific JNK isoform. Here we investigated the role of JNK3 in p75-mediated death of CSN and oligodendrocytes after injury. In culture, JNK3<sup>-/-</sup> mouse oligodendrocytes did not die, while the JNK3<sup>+/+</sup> counterparts underwent apoptosis. This result suggests that JNK3 may also be required for p75-mediated apoptosis in vitro. As the first step to address whether JNK3 is also necessary for the death of injured CSN and oligodendrocytes in vivo, we tested biochemically whether JNK3 is activated following both types of injury. JNK3 activity, which is defined as the JNK activity detected after depleting JNK 1 and 2 from the injured lysates, increased 2-3 fold at 1d after CSN lesion, and at 3d after spinal cord injury. On the other hand, total JNK activity in the same lysates steadily decreased over the course of 5 days. The selective activation of JNK3 and the differential kinetics of JNK3 from JNK1/2 activation suggest that JNK3 is the isoform that is likely to play a critical role in inducing death of CSN and oligodendrocytes in vivo after injury. When JNK3<sup>-/-</sup> mice were subjected to CSN lesion, the proportion of surviving CSN increased to 94% compared to 61% in JNK3<sup>+/+</sup> mice, and similarly after spinal cord injury, the number of apoptotic oligodendrocytes in JNK3<sup>-/-</sup> mice decreased by 31% compared to that in JNK3<sup>+/+</sup>. These results from two different p75-dependent apoptotic paradigms suggest that JNK3 activity is critical in initiating the apoptotic cascade by p75. Expectedly, JNK3 activity is significantly attenuated in p75<sup>-/-</sup> mice compared to p75<sup>+/+</sup> mice after injury. We therefore conclude that p75 is the predominant receptor that activates JNK3 after CNS trauma.

In regard to the mechanisms by which JNK3 induces cell death, we have begun analyzing mitochondrial action after spinal cord injury. In JNK3<sup>+/+</sup> mice, cytochrome C is released into the cytoplasm beginning at 4hr after spinal cord injury, which coincided with the loss of anti-apoptotic Mcl-1 from the cytoplasm. In JNK3<sup>-/-</sup> mice, on the other hand, the cytochrome C release was significantly attenuated, and Mcl-1 remained in the cytoplasm for the entire 5 day post-injury period. We are currently investigating the mechanisms by which JNK3 stabilizes Mcl-1.