Translational Neuroscience

From the bench to the bedside: Taxol for spinal cord injury, dendritic changes in neurons of peri-infarct cortex, neuronal activation and blood flow, normal gut flora and brain development and targeted plasticity for neurological disease

Jason S. Hauptman

Intellectual and Developmental Disabilities Center and Department of Neurosurgery, Geffen School of Medicine at UCLA, Los Angeles, CA, USA

E-mail: *Jason S. Hauptman - jhauptman@mednet.ucla.edu
*Corresponding author

Received: 14 February 11 Accepted: 14 February 11 Published: 17 February 11

Surg Neurol Int 2011, 2:30
This article is available from: http://www.surgicalneurologyint.com/content/1/2/30
Copyright: © 2011 Hauptman JS. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

This article may be cited as:
Hauptman JS. From the bench to the bedside: Taxol for spinal cord injury, dendritic changes in neurons of peri-infarct cortex, neuronal activation and blood flow, normal gut flora and brain development and targeted plasticity for neurological disease. Surg Neurol Int 2011;2:30.
Available FREE in open access from: http://www.surgicalneurologyint.com/text.asp?2011/1/2/78108

MICROTUBULE STABILIZATION REDUCES SCARRING AND CAUSES AXON REGENERATION AFTER SPINAL CORD INJURY

Hellal F, Hurtado A, Ruschel J, Flynn KC, Laskowski CJ, Umlauf M, Kapitein LC, Strikis D, Lemmon V, Bixby J, Hoogenraad CC, Bradke F.

Article: Science. 2011 Jan 27. [Epub ahead of print]

Key points
1. The fibrotic scar is a major impedence to axonal recovery following spinal cord injury (SCI).
2. Microtubule dynamics is critical to scar formation.
3. Taxol, an FDA-approved anticancer agent, stabilizes microtubules and prevents their disassembly.
4. In SCI models, Taxol treatment (at doses well below those used in cancer therapeutics) results in decreased scar formation, enhanced axonal regeneration and improved functional recovery.

The key concept explored in this paper is whether the stabilization of microtubules following spinal cord injury (SCI) could help to enhance axon regeneration. Unlike vehicle-treated animals, Taxol-treated animals showed lower levels of molecules that comprise the fibrotic scar without changing the injury size, the ability of local astrocytes to isolate the injured cells or local cell growth/automated cell death.

Upon examination of tissue samples from the injury site, the authors found that Taxol appears to inhibit the TGF-β pathway, a molecular pathway that is usually upregulated and promotes fibrotic scarring after SCI. One of the downstream effector molecules of the TGF-β pathway, Smad2, was inhibited from being transported to the cell nucleus by Taxol (a process that would otherwise occur along the microtubules). Taxol was also found to have other effects as well: it directly inhibited the production of fibronectin (a component of the fibrotic scar) by meningeal cells and reduced the amount of molecules that inhibit axon growth (these are called chondroitin sulfate proteoglycans).
The authors then used these findings to determine whether Taxol treatment could enhance axon regeneration. They shifted focus to dorsal root ganglion (DRG) neurons and injured the peripheral axon (in this model, after injuring the peripheral axon, the CNS axon will actually grow only if no scar is present). As one would expect, they found that Taxol-treated animals showed much greater degrees of axon regeneration (three-quarters of vehicle-treated animals did not regenerate at all). In another model of CNS injury, Taxol was applied following hemisection of the Raphe-spinal tract. Again, Taxol treatment resulted in a greater number of serotonergic fibers (presumably from the Raphe) caudal to the lesion 4 weeks after injury. These fibers were characterized by the appearance of growth cones instead of retraction bulbs (what is normally found after injury).

To translate this to functional recovery, the authors found that Taxol-treated animals improved their locomotion well beyond the recovery in vehicle-treated animals. Both Taxol-treated animals and controls improved in the first 4 weeks, after which the controls reached plateau. Taxol-treated animals, however, continued to improve over the next 4 weeks. This study is a great example of exploiting FDA-approved therapies (for other conditions) in novel ways to address CNS injury. The authors show that Taxol results in a beneficial effect on recovery from SCI by stabilizing microtubules and inhibiting fibrotic scar formation.

**ABSENCE OF LARGE-SCALE DENDRITIC PLASTICITY OF LAYER 5 PYRAMIDAL NEURONS IN PERI-INFARCT CORTEX**

Mostany R, Portera-Cailliau C.

Article: J Neurosci. 2011 Feb 2;31(5):1734-8.

**Key points**

1. How neurons in areas surrounding cortical injury contribute to functional recovery is controversial, with no clear answer as to how neurons reorganize
2. In this study, the authors visualize the apical dendrites of layer 5 pyramidal neurons in peri-infarct cortex to see how they change
3. These apical dendrites undergo a reduction in branching and overall length following injury. After looking at the dendritic tips, it is apparent that this reduction in length is due to tip retraction and a reduction in overall terminal branch length
4. These results suggest that plasticity is not occurring at the level of apical dendrite organization in peri-infarct cortex

An unresolved problem in the field of neuronal injury pertains to understanding how spared neurons reorganize or rewire to compensate for functional loss. Existing evidence has proved controversial, with some studies suggesting that surviving neurons grow new dendrites and branches while others show the opposite. Even though work has suggested that the areas surrounding the injury are significantly rewired, it is not clear how that translated to dendritic remodeling. In this study, the authors use a technique called in vivo two-photon microscopy (a way of visualizing rapid microscopic changes in neurons of intact animals) in animals following middle cerebral artery (MCA) occlusion to examine dendritic reorganization of layer five pyramidal neurons in cortical regions surrounding the infarct. They repeatedly imaged 11 of these layer five neurons over the course of 3 months following the stroke.

The authors found that after the stroke, the apical dendrites of the neurons showed reduced branches (with more proximal fifth- and sixth-order branches affected more than the most distal seventh- to ninth-order branches). Interestingly, this process is not quick—the branch reduction takes at least 30 days after stroke to occur. Not only was there less branching, but the apical dendrites were also found to be shorter overall. Especially pronounced was a shortening of the most proximal dendrites (first- and second-order). This effect was quicker than the reduction in branching.

So the question then becomes, why are the dendrites shorter? Is it due to retraction of the dendritic tips or shrinkage of the entire dendrite? To answer this question, the authors looked at more than 40 dendritic tips at high resolution to see how they changed over time. First, they found that before the stroke, the length of the tips changed very little. In the first few days following stroke, the tips began to shorten. When the authors compared the change in total length of the distal branches to the change in the distal tips, they found that the overall change in length of the branch comprised of about 60% retraction of the tip and 40% shrinkage of the entire dendritic branch. Interestingly, there was no correlation between the distance of the neuron from the infarct and the degree of dendritic shrinkage, such that all neurons in the peri-infarct area were affected equally.

This paper is particularly exciting for two reasons. First, it highlights an innovative technique that allows for imaging dynamical activity of the dendrites in intact animals (compare to the traditional Golgi stain, which gives a “snapshot” of the neuron in brain slices). Second, it provides a comprehensive answer to what it happening to spared neurons in an area surrounding injury. This work suggests that changes in the complexity of layer 5 pyramidal neuron apical dendrites do not likely contribute to functional recovery after injury. This does not mean that changes are not occurring at the basal dendrites or at the molecular level. It does, however, suggest a possible target for therapy to enhance recovery following injury (i.e., therapies that would enhance dendritic growth).
WHAT DOES LOCAL FUNCTIONAL HYPEREMIA TELL ABOUT LOCAL NEURONAL ACTIVATION?

Jukovskaya N, Tiret P, Lecoq J, Charpak S.
Article: J Neurosci. 2011 Feb 2;31(5):1579-82.

Key points
1. Blood oxygen level-dependent (BOLD)-functional magnetic resonance imaging (fMRI) is a useful tool for delineating areas of the cortex that are important for language or movement prior to surgery.
2. BOLD signals represent increases in blood flow to cortical areas occurring as the subject performs a task; controversy has existed over whether increases in blood flow necessarily represent neuronal activation.
3. In this paper, the authors show that the correlation between blood flow and neuronal activation is not always positive. That is, in some situations, blood flow may increase while neuronal activation is low.
4. This means that the BOLD signal cannot be accepted as a de facto marker of neuronal activation under all circumstances.

BOLD-fMRI is a technique that allows one to image activation of certain brain areas while the subject performs a task (i.e., maps important functional brain areas). This “activation” is not a signal generated directly by neurons, but instead is generated by increases in local blood flow. A crucial assumption in BOLD-fMRI is that increases in cerebral blood flow (CBF) represent activation of neurons. This assumption is still controversial. This is an idea that has been discussed in From the Bench to the Bedside before (July 2010). In this study, the authors look at the olfactory bulb glomeruli to see if neuronal activation (visualized as an increase in calcium levels within the presynaptic neuron that lead to glutamate release) by odor is linked to increases in local blood flow (hyperemia). Like the previous article, the researchers used in vivo two-photon microscopy. But instead of using it to look at the structure of neurons, they used it to look at calcium signals within the neurons by employing fluorescent calcium dyes.

The authors started by showing that in olfactory glomeruli that did not adapt to odors (adaptation refers to the common phenomenon whereby after a sensory neuron is exposed to a stimulus for a long period of time, it requires stronger stimulation in order to activate), calcium signals and hyperemia both increased as odor intensity increased. Adaptation is a common phenomenon and one that’s quite useful, for example during vision (in areas where ambient light is brighter the retina requires more light to be stimulated). In olfactory glomeruli that did show adaptation at high odor intensities; however, the relationship between activation and blood flow was different. During adaptation, calcium signals dropped to levels below weak stimulation, since the neurons were resistant to activation. Vascular responses, on the other hand, increased, suggesting the relationship between neuronal activation and hyperemia became uncoupled.

Using the olfactory glomeruli model, the authors show that the relationship between blood flow and neuronal activation is complex. In the absence of adaptation, the responses are directly related (as activation increases, blood flow increases). When adaptation occurs, these responses become decorrelated (blood flow no longer necessarily represents activation). What is not clear is whether these findings can be extrapolated to other brain regions that clinicians test with BOLD-fMRI, though there are some indirect suggestions that this may be the case. Regardless, this stresses the interpretation of the BOLD signal with caution, as hyperemia is not necessarily equivalent to neuronal activation.

NORMAL GUT MICROBIOTA MODULATES BRAIN DEVELOPMENT AND BEHAVIOR

Heijtz RD, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S.
Article: Proc Natl Acad Sci U S A. 2011 Jan 31. [Epub ahead of print]

Key points
1. Normal gut microbiota have been shown to influence a variety of systemic processes.
2. In this study, the authors examine the role of normal gut microbiota on brain development and function.
3. Mice that were raised in the absence of any gut microbiota showed increased locomotion and decreased anxiety that were reversed when mice were raised in the presence of normal gut flora.
4. Mice without normal gut flora also showed changes in monoamine neurotransmitter turnover, expression levels of genes involved in synaptic plasticity and other cellular processes, and protein markers for synapse formation.

When considering brain development, the usual thoughts that come to mind pertain to concepts such as neurogenesis, neuronal migration and programmed cell death. This paper stood out because it examines a novel concept modulating brain development, the “normal” gut microbiota. The authors chose to study this because: (1) it is known that external, environmental signals influence brain development and function and (2) normal gut flora has been linked to a variety of systemic processes. The authors started by taking adult mice that were raised to be completely germ-free including gut flora (GF mice) and mice that were specific pathogen free but had normal gut flora (SPF mice) and subjecting them to behavioral testing. They found that the GF mice showed behavior...
consistent with increased motor activity and reduced anxiety compared to the SPF mice. Interestingly, when GF mice were raised in the presence of the same bacteria that SPF mice contained (i.e., gut flora), these behavioral differences disappeared and the new GF mice were no different than the SPF mice. This only occurred when the GF mice were raised in the environment that allowed for exposure to the bacteria—when adult GF mice were exposed their behavior did not change.

To investigate the neurochemical source of behavior changes in the GF mice, the authors examined neurotransmitter levels to see if there were changes in monoamines (neurotransmitters that have been linked to anxiety behavior). They found that the GF mice had significantly greater turnover of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in the striatum compared to SPF mice. “Turnover” was described as the ratio of the neurotransmitter metabolite to the neurotransmitter itself; therefore, higher turnover suggests lower neurotransmitter levels and/or higher metabolite levels. To further examine the circuit involved in anxiety, the authors looked at the expression of genes involved in the learning (i.e., synaptic plasticity) of anxiety, including brain-derived neurotrophic factor (BDNF) and nerve growth factor-inducible clone A (NGFI-A). They found that levels of expression of these genes were reduced in the cortex, striatum and hippocampus of GF mice. Changes in dopamine receptor expression were noted as well.

Next, the authors looked at a vast array of gene expression patterns in GF and SPF mice. The found differential expression of a wide variety of genes involved in metabolism, synaptic long-term potentiation (LTP, a form of cellular learning), steroid hormone metabolism and the cyclic adenosine monophosphate (cAMP) signaling pathway (a common signaling pathway that the cell uses to respond to a variety of extracellular stimuli) in the GF mice compared to the SPF mice. These changes were noted in the cortex, hippocampus and striatum. Furthermore, the authors found that the GF mice had significantly higher levels of proteins linked to the formation of synapses (PSD-95 and synaptophysin).

In this study, mice without gut flora had greater locomotive and exploratory behavior and reduced anxiety. They noted changes in gene expression and neurotransmitter turnover that could (at least in part) explain this behavior. Taken together, this paper provides evidence that environmental stimuli (in this case normal gut flora) may have a significant impact on brain development and function later in life. That being said, the mechanism by which gut flora influences this is not readily clear. Perhaps it is through the modulation of gut hormones, stress hormones or nutrient signaling.

**REVERSING PATHOLOGICAL NEURAL ACTIVITY USING TARGETED PLASTICITY**

Engineer ND, Riley Jr, Scale JD, Vrana WA, Shetake JA, Sudanagunta SP, Borland MS, Kilgard MP.

Article: Nature. 2011 Feb 3;470(7332):101-4. Epub 2011 Jan 12.

**Key points**

1. One mechanism by which vagal nerve stimulation (VNS) works is through enhancement of synaptic plasticity (“neuronal learning”), presumably through the release of a variety of neurotransmitters in multiple brain regions.

2. In this study, the authors use VNS to reverse the pathological plasticity that is present in a rat model of tinnitus.

3. VNS paired with multiple pure tones with frequencies that span the range of rat hearing reverses pathological findings in noise-exposed (i.e., tinnitus) rats, including restoration of normal auditory cortex representation, improved frequency discrimination and normalization of function of auditory neurons.

Synaptic plasticity (“neuronal learning”) can be a beneficial mechanism, particularly when related to learning behavior or generating memory. It can also be hijacked by pathological processes, as occurs in conditions such as chronic pain or tinnitus. It stands to reason that one way to treat these conditions would be to reverse the aberrant synaptic plasticity that led to the condition in the first place. In this study, the authors use VNS as a way of modulating plasticity. It is known that VNS enhances plasticity, which is likely related to changes in neurotransmitters throughout the brain. The authors used VNS and audio tones to attempt to reverse the pathological plasticity present in rats suffering from tinnitus.

First, the authors paired VNS with one of two different tones 300 times a day for 20 days in normal rats. They found that this procedure resulted in a ~70-79% increase in the number of sites within the primary auditory cortex dedicated to tones near the frequency that was delivered during the VNS trial compared sham controls. This is a result of enhanced neural plasticity within the auditory cortex; more neurons have learned to respond specifically to tones near the learned frequency.

In the next set of experiments, the authors created a rat model of tinnitus. In this model, rats were exposed to loud, high-frequency noise. This results in rats that are less able to distinguish specific auditory frequencies, have auditory cortices that are over-represented by cells that respond to middle frequencies, and have auditory neurons that are more excitable and more likely to fire in synchrony. Many auditory cortex neurons responded to

Surgical Neurology International 2011, 2:30

http://www.surgicalneurologyint.com/content/1/2/30
a wider array of frequencies than normal (this is termed the “tuning curve”, which is widened). Furthermore, the severity of changes in auditory cortex and tuning curve paralleled the severity of impairment in frequency discrimination. These changes have been used as a behavioral correlate for tinnitus (since it is not possible to measure tinnitus in other ways).

The authors then set out to show that using VNS, one could reverse these pathological auditory changes by pairing VNS with random pure tones that spanned the entire frequency range of the rat. To do this, tones were paired with VNS 300 times a day for 18 days in rats that were pathologically exposed to noise (and suffered from the correlate of tinnitus). They found that VNS-treated rats showed improvements in tone discrimination that lasted well beyond the duration of therapy. This confirmed long-lasting changes in this pathological plasticity, suggesting that it had been successfully reversed. Three weeks after the end of therapy, the authors examined the auditory cortex and found improvement in the representation of tones in the auditory cortex as well as narrowing of the auditory cortical neurons’ tuning curves. Furthermore, the pathological changes in neuron excitability and synchronization were reversed by VNS as well.

This is a particularly exciting paper because it demonstrates a novel method by which clinicians could potentially treat patients with disorders that stem from pathological plasticity, such as chronic pain or tinnitus.