Promotion of neuroplasticity by modifying perineuronal nets using polysialic acid

Louise Adams, Yi Zhang, John Priestley, Xuenong Bo

Centre for Neuroscience and Trauma, Blizard Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, 4 Newark Street, London, E1 2AT, UK

L.Adams@qmul.ac.uk

In the central nervous system (CNS), certain populations of neurons are surrounded by a specialised form of extracellular matrix called the perineuronal net (PNN). Formed of a network of proteins, including a high concentration of chondroitin sulphate proteoglycans (CSPGs), the PNN is anchored to the cell membrane by the neural cell adhesion molecule (NCAM) and acts to limit neuroplasticity in the adult CNS.

Unpublished data from our lab suggests there is a negative correlation between PNN formation and the expression of polysialic acid (PSA) during early postnatal development of the rat spinal cord. PSA, a homopolymer of α2-8-linked *N*-acetylneuraminic acid is attached to NCAM by the enzyme polysialyltransferase (PST). Predominantly found during postnatal development, PSA is thought to decrease the adhesive force of NCAM and has been shown to increase intercellular spacing by up to 15 nm. We believe that in addition to this, PSA can also modify the neuronal extracellular matrix, in particular the PNN.

We have developed a lentiviral vector carrying the PST transgene (LV/PST) that has previously been shown to overexpress PSA *in vivo*, when compared with a control vector carrying the transgene for green fluorescent protein (LV/GFP). In this study, we injected either LV/PST or LV/GFP into the lumbar spinal cord of adult rats. After 6 weeks, animals were sacrificed by transcardial perfusion and spinal cords were prepared for double labeled immunohistochemistry, using antibodies against PSA and components of the PNN.

Preliminary results demonstrate that immunoreactivity for two markers of the PNN, Wisteria floribunda agglutinin and cartilage link protein 1, were diminished in the regions of PSA overexpression. Although it appears the PNNs have been degraded at these regions, further immunohistochemistry using antibodies directed against individual CSPG core proteins is required before we can form any firm conclusions.

Supported by the International Spinal Research Trust

Novel interactions between the c-Jun and Notch Signalling pathways regulate the Schwann cell response to peripheral nerve injury

Daniel K. Wilton[1], K. Malgapo[1], J. Shen[2], D. Meijer[3], L. Feltri[4], L. Wrabetz[4], A. Behrens[5] R. Mirsky[1], K. R. Jesssen[1]

[1]Department of Cell and Developmental Biology, University College London, UK
[2]Ctr. For Neurologic diseases, Brigham and Women's hospital, Harvard Med. Sch, Boston, MA, USA
[3]Dept of cell biology and genetics, Erasmus MC, Rotterdam, Netherlands
[4]School of Medicine and Biomedical Sciences, University of Buffalo, New York, USA
[5]Mammalian Genetics Laboratory, London Research Institute, CRUK, London WC2A 3LY, UK

daniel.k.wilton@btinternet.com

We have previously shown that in Schwann cells the transcription factor c-Jun acts as a master regulator of Wallerian degeneration and is required for successful repair following nerve injury (Wilton et al., (2009) Glia, 57 (13) S158-S158); Latouche et al., (2009) Glia, 57 (13), S158 – S158). In this study we identify a novel role for c-Jun in the activation of Notch signalling in the denervated Schwann cell. We find that c-Jun is required to activate Notch signalling, leading to upregulation of the BHLH protein Hes1. Hes1 then plays two functions in the denervated cell, promoting myelin breakdown and acting as part of a negative feedback loop to reduce c-Jun levels. As a result of this, ablating Notch signalling specifically in Schwann cells acts to increase c-Jun levels. We show that this upregulation of Schwann cell c-Jun accelerates axon outgrowth, target re-innervation and remyelination by generating a cell, which results in a more rapid functional recovery. These results identify novel functional links between the c-Jun and Notch signalling pathways. They also show that not only is Schwann cell c-Jun necessary for successful nerve regeneration, but that nerve repair can be improved by enhancing normal c-Jun signalling.

Session VI: Spasticity, bladder/bowel and sexual function
Chair: Geoffrey Raisman

New perspectives for the treatment of spasticity

Laurent Vinay, Rémi Bos, Pascale Boulenguez, Hélène Bras, Cécile Brocard, Dorothée Buttigieg, Florian Gackière, Georg Haase, Sylvie Liabeuf, Karina Sadlaoud

Institut de Neurosciences de la Timone, Centre National de la Recherche Scientifique (CNRS) & Aix-Marseille Université, Marseille, France

laurent.vinay@univ-amu.fr

A decrease in synaptic inhibition - disinhibition - appears to be an important substrate in several neuronal disorders, such as spinal cord injury (SCI) and neuropathic pain (Boulenguez et al., 2009). Glycine and GABA are the major inhibitory transmitters in the spinal cord. An important emerging mechanism by which the strength of inhibitory synaptic transmission can be controlled is via modification of the intracellular concentration of chloride ions ($[Cl^-]_i$) to which receptors to GABA/glycine are permeable. Briefly, a low $[Cl^-]_i$ is a pre-requisite for inhibition to occur and is maintained in healthy neurons by cation-chloride co-transporters (KCC2) in the plasma membrane, which extrude Cl^-.

We showed that KCC2 is down-regulated following SCI in rats, particularly in motoneuron membranes, thereby depolarizing the Cl^- equilibrium potential and reducing the strength of postsynaptic inhibition (Boulenguez et al., 2010). This result can account for the hyperexcitability of spinal reflexes and reduced inhibition which are commonly associated with spasticity after SCI. Blocking KCC2 in intact animals by intra-thecal injection of DIOA reduces the rate-dependent depression (RDD) of the Hoffmann reflex as observed in spasticity. RDD is also decreased in KCC2-deficient mice.

Given the critical role of KCC2 in regulating the strength and robustness of inhibition, identifying tools that may increase KCC2 function and hence restore endogenous inhibition in pathological conditions is of particular importance. We showed that the early decrease in KCC2 after SCI is prevented by pre-treatment with the BDNF-sequestering TrkB/Fc chimera protein. Conversely, two weeks after SCI, BDNF up-regulates KCC2 and restores RDD (reduces spasticity). More recently, we demonstrated that activation of $5\text{-}HT_{2A}$ receptors to serotonin hyperpolarizes the reversal potential of IPSPs (E_{IPSP}) in spinal motoneurons, increases the cell-membrane expression of KCC2 and both restores endogenous inhibition and reduces spasticity after SCI in rats. Upregulation of KCC2 function by targeting $5\text{-}HT_{2A}$ receptors therefore has therapeutic potential in the treatment of neurological disorders involving altered chloride homeostasis. These results open new perspectives for the development of therapeutic strategies to alleviate spasticity and chronic pain after SCI.

References

1. Boulenguez P, Vinay L (2009) Strategies to restore motor functions after spinal cord injury. *Curr Opin Neurobiol* 19:587-600.
2. Boulenguez P, et al. (2010) Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat Med* 16:302-307.

Supported by grants to L. V. from the International Spinal Research Trust (ISRT), the Christopher and Dana Reeve Foundation, the French Agence Nationale pour la Recherche, the French Institut pour la Recherche sur la Moelle épinière et l'Encéphale. Salary support: ISRT to F.G., Institut National de la Santé et de la Recherche Médicale (INSERM) to H.B. and the Association Française contre les Myopathies (Grant 13912) to K.S.

Gene delivery of chondroitinase ABC promotes functional repair following contusion injury at thoracic or cervical level

Nicholas D. James, Jessie R. Shea, Karen D. Bosch, Katalin Bartus, John H. Rogers, Stephen B. McMahon, Bernard L. Schneider, Elizabeth M. Muir, Elizabeth J. Bradbury

Regeneration Group, Wolfson Centre for Age-Related Diseases, King's College London, London SE1 1UL, UK

nicholas.n.james@kcl.ac.uk

Spinal cord extracellular matrix is densely packed with growth inhibitory chondroitin sulphate proteoglycans (CSPGs), which become more abundant after injury. Thus, matrix modification has become a leading experimental strategy for promoting repair following spinal cord injury. Despite the beneficial effects that have been achieved by digesting CSPGs with the bacterial enzyme chondroitinase ABC (ChABC), the potential for achieving long term efficacy in traumatic injuries that mimic a human spinal cord injury has not yet been realised. Gene therapy offers a route to achieving stable continuous delivery of ChABC and therefore, here we deliver genetically modified ChABC via a lentiviral vector (LV-ChABC) to the adult rat spinal cord and assess the efficacy of chronic gene delivery using a spinal contusion injury model. Contusion injury represents the most common form of spinal cord injury in humans and, therefore, provides a clinically relevant tool for assessing the efficacy of potential therapeutic interventions. Adult rats received a moderate severity thoracic (T10) contusion injury and LV-ChABC or a control LV-GFP was immediately injected rostral and caudal to the injury site. We demonstrate prolonged and widespread CSPG degradation with LV-ChABC and, using both behavioural and electrophysiological outcome measures, we show improved function in animals treated with LV-ChABC. We saw a dramatic increase in spinal conduction through the injury site as well as a significant improvement in performance on the horizontal ladder test. Using an additional electrophysiological technique we also saw evidence of plastic changes in the form of reorganisation of spinal circuitry below the level of the injury. In order to enhance the potential clinical applications of this study we have now also assessed the effects of LV-ChABC in a moderate severity cervical (C5) contusion injury. Approximately 50% of all human spinal cord injuries occur at the cervical level making this injury model of particular clinical relevance as well as allowing us to assess a number of additional functional outcomes such as forelimb grip-strength, sensory and motor function during sticky-tape removal, and proprioception using the inclined plane. We again saw significant improvements in the horizontal ladder task as well as a striking increase in spinal conduction; in addition there were also modest improvements in forelimb grip-strength. Thus, we demonstrate the potential advantages of gene delivery of ChABC for achieving sustained and widespread CSPG degradation and that this is associated with functional improvements following contusion injury.

Supported by: The U.K. Medical Research Council, the International Spinal Research Trust, the Darwin Trust of Edinburgh and the International Federation for Research in Paraplegia

Wow, I can make 3-4 times more money than a high-yield savings account! (Interviewee: 33 years old, male, entrepreneur)
According to news from the relevant media on April 20, Dogecoin founder Jackson Palmer suddenly set his Twitter account to a protected privacy status and deleted many videos about cryptocurrencies. It’ s unclear what exactly happened, but on April 2nd Palmer stated
Each ABLE account includes account information, account holder, account number, password, account binding email address, savings currency and balance. After the ABLE account is processed, users can transfer or make withdrawals through the ABLE free account and use a variety of products based on smart contracts.
Savings refers to the currency-based savings products, according to the investment currency, also divided into BTC, USDT two products, according to the investment cycle, taking into account the user's complex requirements for asset flows, divided into 90 days of regular, 30 days of regular, demand savings three types.
Last month, Robinhood launched a savings and checking account for its U.S. customers, offering users a "free, free, free and no surprise" account that pays 3 percent interest on each deposit in the account.
July 18 (Xinhua) -- Hackers have hacked Elon Musk's Twitter feed over the past two days and posted scams involving Bitcoin, according to NewsBTC. The account is now back to normal, and when a Twitter fan asked him "Where's my bitcoin," Elon Musk quipped, "Excuse me, I only sell Dogecoin." Minutes after his comments, he posted a picture on Twitter showing a "Dogecoin Standard" sandstorm sweeping through a city labeled the "global financial system."
It is reported that users need to register a current and savings account at the same time to enjoy a 2.69 percent yield, and this expected return only until the end of the year, may also change with the Federal Reserve interest rate cut and other environmental factors. Users who do not have a current account expect savings yield to fall to 2.43%
In traditional banking institutions, people have a checking account to complete all kinds of daily consumption and transactions, while another savings account holds cash that will not be spent in the short term.
Stash account: is a regular Kusama account, also known as a savings account, characterized by a relatively large address balance, in order to keep assets safe offline.
Restaurants in Maryland accept e-money dogecoin, MyFoxDC reported. Iron Rail Restaurant in Mount Savage, Maryland, officially accepts Dogecoin. Owner Terry Ii says the use of dogecoin has benefited restaurants, including the need not to pay for credit cards.
Pillar is the enterprise supplementary old-age insurance, that is, the use of personal account savings accumulation market-oriented operation model, as long as the embodiment of the savings function of old-age insurance.
Unless you have a Tether international account. Of course, this will not affect your savings. But Forbes independent writer Frances.
Dogecoin account asking who should be the cr

yptocurrency’ s next CEO an absurd
Dogecoin founder Jackson Palmer commented on Twitter.
Step: Transfer EOSC from your savings account to your pool account, enter the quantity you need to transfer, and click OK.
As a businessman, you can save $2,800 a month, which you can put into a savings account at a 1% interest rate.
Dynamic . . . Bankers express concern about Robin Hood's new savings account, calling it 'deceptive'
Derivatives exchange LedgerX launches CFTC-regulated Bitcoin savings account with annual returns of 16%
The Polish Savings Bank will work with Ukinform in the coming days to launch a blockchain solution for its client files, and the Polish Savings Bank will use Brainform's Trudatum to provide blockchain-publishing paperwork for its approximately 5 million account holders.
The new service is designed to take into account the operating habits of LedgerX's existing customers and is expected to attract a large number of users. It doesn't work much differently from a normal savings account. However, the product may be more attractive to Bitcoin hodlers, after all, the meaning of this savings account is to let users put their digital currency in and wait for the proceeds. As a result, LedgerX says the savings service is better suited to long-term holders of Bitcoin.
On March 3rd Musk praised Dogecoin: "It's so cool, dog money is probably my favorite cryptocurrencies." "And change the account profile from "Dog Coin."
Peculium is latin for "property", meaning having a child savings account - that's exactly what Peculium is targeting. Their tokens are called P?cule (PCL), also in Latin, which stands for "savings". As a result, Peculium is a savings platform for individuals, brokers and financial institutions and serves as a bridge between traditional capital markets and the nascent cryptocurrencies market. Their AI uses algorithmic trading and other means to increase the user's savings account. It also uses big data to predict future market movements. They are currently in the ICO phase and the token sale will be completed within 20 days.
How can the soV functionality of cryptocurrencies actually be of little use? Proponents of this argument argue that as long as users can convert SoV functionality into more practical switching media (MoE) functionality, SoV does its job: store value. MoE enables more frequent small transactions. It's like having a savings account and a checking account at the same time: users keep their money in a savings account and often transfer some of it from the savings account to a checking account, making it easier to spend.
February 16 (UPI) -- On the advice of Larry Kudlow, director of the National Economic Council, he proposed creating an ordinary savings account that would include pensions, health care and education savings in a single account. Taxpayers can fund their accounts tax-free and invest them as they wish. As long as the money continues to be invested in the savings account, investors can trade digital currencies such as Bitcoin without paying taxes. (beincrypto)


Profit/Loss
Target Price
Liquidation Price
Side:
Long
Short
Quantity
5894
Entry Price
5894
Exit Price
6189
Leverage
10
Margin
0.1016
Entry Value
-0.9999
Exit Value
-0.9523
Profit/Loss
0.0476
Profit/Loss %
4.76%
ROE %
47.62%
All currency units denominated in XBT
Position: —
Contract: XBTUSD

A novel gene therapy approach for reprogramming intraspinal inflammation

Phillip G. Popovich

Department of Neuroscience, Wexner Medical Center at The Ohio State University, Center for Brain and Spinal Cord Repair, USA

phillip.popovich@osumc.edu

An inevitable consequence of traumatic spinal cord injury (SCI) is the accumulation of macrophages within and nearby the site of injury. These cells persist indefinitely, closely apposed to intact cells and axons. The precise role played by macrophages is controversial. Recently, we showed that most intraspinal macrophages adopt a neurotoxic "M1" phenotype[1]. M1 macrophages cause axonal "die-back" *in vitro* and *in vivo* and may also contribute to protracted cell death[1,2]. Although some newly activated macrophages become non-toxic pro-regenerative "M2" macrophages, this phenotype is not maintained by cells in/nearby the site of injury[1]. Instead, undefined factors in the injury microenvironment (or lack thereof) polarize macrophages toward an M1 phenotype[1]. A goal of our current research program is to learn how to reprogram the natural course of macrophage activation such that the ratio of M1:M2 macrophages is reduced. One way to accomplish this is to manipulate the composition of the extracellular milieu such that newly activated monocytes or microglia differentiate into M2 macrophages. Preliminary data will be presented showing that systemic post-injury injection of a novel adeno-associated virus (AAV9)[3] engineered to produce interleukin-4 (IL-4) augments M2 macrophage activation *in vivo*. Ongoing studies will determine whether functionally significant axon regeneration can be achieved after SCI by simultaneously manipulating neuron extrinsic (macrophages) and intrinsic (e.g., *Pten*) barriers to axon regeneration.

References

1. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of Two Distinct Macrophage Subsets with Divergent Effects Causing either Neurotoxicity or Regeneration in the Injured Mouse Spinal Cord. J Neurosci 29: 13435-13444.
2. Horn KP, Busch SA, Hawthorne AL, van Rooijen N, Silver J (2008) Another Barrier to Regeneration in the CNS: Activated Macrophages Induce Extensive Retraction of Dystrophic Axons through Direct Physical Interactions. J Neurosci 28: 9330-9341.
3. Foust KD, Nurre1 E, Montgomery CL, Hernandez A, Chan CM & Kaspar BK, Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes (2009) Nat Biotechnol. 27(1):59-65.

Supported in part by the International Spinal Research Trust, The Craig H. Neilsen Foundation and the Ray W. Poppleton Endowment

Collaborators: Kristina A. Kigerl, Jodie C. Hall, Brian K. Kaspar, Kevin D. Foust, Zhigang He

Functional roles of chondroitin sulfate glycosaminoglycans in axon regeneration

Linda C. Hsieh-Wilson

Division of Chemistry and Chemical Engineering, California Institute of Technology and the Howard Hughes Medical Institute, Pasadena, CA 91125, USA

lhw@caltech.edu

A major obstacle to functional recovery after CNS injury is the inhibitory environment encountered by regenerating axons. Chondroitin sulfate (CS) polysaccharides and their associated proteoglycans (CSPGs) are the principal inhibitory components of the glial scar, which forms after neuronal damage and acts as a barrier to axon regeneration. It is well established that the inhibitory activity of CSPGs is primarily derived from their CS chains, as chondroitinase ABC (ChABC) treatment promotes axon regeneration, sprouting, and functional recovery after injury *in vivo*. However, the mechanisms by which CS polysaccharides inhibit axon re-growth are not well understood, limiting the development of molecular approaches to counteract CSPGs. We will describe the synergistic application of organic chemistry and neurobiology to understand how CS polysaccharides contribute to neuronal growth and regeneration. By taking advantage of our ability to synthesize defined oligosaccharides, we demonstrate that a specific sugar epitope on CSPGs, chondroitin sulfate-E (CS-E), potently inhibits axon growth. CS-E functions as a protein recognition element to engage receptors, including the transmembrane protein tyrosine phosphatase PTPσ, thereby triggering downstream signaling pathways that inhibit axon growth. Masking the CS-E motif using a CS-E-specific antibody reverses the inhibitory activity of CSPGs and stimulates axon regeneration *in vivo*. Targeting specific sugar epitopes using antibodies, small molecules, or other approaches may offer a more stable, selective, and less immunogenic alternative to ChABC. Given that CS-E appears to interact with multiple receptors, strategies that block the sulfated CS-E epitope may also prove more effective at neutralizing CSPGs than targeting individual receptors or pathways.

Supported by NIH R01 GM093627-03, Roman Reed Spinal Cord Injury Research Fund, and Christopher & Dana Reeve Foundation.

AAV vector allows widespread and long-term secretion of chondroitinase ABC in rat CNS

João Nuno Alves [a,b], Melissa R. Andrews [a], Anneliese Ward [b], Nicholas Michelmore [b], Joost Verhaagen [c], Elizabeth Moloney [c], Roger J. Keynes [b], James W. Fawcett [a], John H. Rogers [b], **Elizabeth M. Muir [b]**

[a] Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge CB2 0PY, UK
[b] Department of Physiology Development and Neuroscience, University of Cambridge, Downing St, Cambridge, CB2 3EG, UK
[c] Laboratory for Neuroregeneration, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105BA Amsterdam, Netherlands

emm1@mole.bio.cam.ac.uk

The bacterial enzyme chondroitinase ABC has been shown to promote axon regeneration following spinal cord injury in many models and in three species. It is therefore a promising strategy for the treatment of SCI. However, repeated injections, which would likely be required for human treatment, would increase the risk of causing further trauma and infection, therefore gene therapy is a desirable alternative route of administration. We have modified the bacterial chondroitinase gene to achieve efficient secretion of active chondroitinase from mammalian cells, as previously demonstrated with lentiviral vectors both *in vitro* and *in vivo*, assessed by staining for the products of digestion (stub antibody 2B6). We then inserted the gene into AAV vectors, since these vectors are currently considered the safest for the treatment of patients. AAV-GFP and/or AAV-chondroitinase vectors were injected into adult rat cortex and expression profiles were examined 4 weeks later. Both AAV5 and AAV8 gave efficient transduction around the injection site. GFP was expressed by many neurons and their axons, including the corticospinal tract. Chondroitinase was expressed strongly and widely as indicated by 2B6 staining. Injections into vibrissal-motor cortex led to chondroitinase secretion in the midbrain, including the substantia nigra. Injections that spread into adjacent areas of cortex and hippocampus led to additional chondroitinase secretion which in some areas extended beyond the visible GFP-positive fibres, probably because it is efficiently secreted from very thin, terminal arborisations from some neuronal types. At 12 weeks post-injection, chondroitinase expression was still widespread.