# Two methods of biomarker discovery: applications in neuropathic pain and pharmacotherapy

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## Abstract

Biomarkers have potential utility in the treatment of pain as diagnostics and for quantification of drug efficacy and safety. A qualified biomarker will capture overlapping disease mechanisms and will be responsive to treatment. The necessity for these strict requirements renders it difficult to discover new biomarkers, particularly one that is reliable, practical and non-invasive, and simple for routine utilisation. This thesis demonstrates that two approaches may be useful to overcome these challenges: bottom-up and top-down biomarker discovery and development. Current animal models of neuropathic pain are inadequate to develop biomarkers as they only cover 'no pain' and 'high pain': not the heterogeneity that exists between these extremes. Therefore, a novel rat model of graded neuropathic pain was developed by advancing the existing chronic constriction injury model. Sciatic nerve and subcutaneous chromic gut sutures were varied, resulting in 'dose-dependent' behavioural allodynia. Allodynia was correlated with microglial activation marker expression in the ipsilateral lumbar dorsal horn of the spinal cord, suggesting that changes in behaviour are associated with disease mechanisms. A literature review of the pathophysiological mechanisms of pain, filtered by the criterion for accessible biomarkers, revealed that the peripheral immune system was the ideal target for the bottom-up approach. As such, the graded model was then used to explore peripheral immune mechanisms in order to begin the process of construct validation of potential neuropathic pain biomarkers. It was demonstrated that peripheral immune cells significantly contribute to chronic constriction injury-induced allodynia, as adoptive transfer of splenocytes or peripheral blood mononuclear cells from high pain donors to low pain recipients potentiates allodynia. Intrathecal transfer of high pain immune cells to low pain recipients potentiated allodynia, confirming that infiltrating immune cells are not passive bystanders, but actively contribute to nociceptive hypersensitivity in the lumbar spinal cord. The graded transcriptome of dorsal horn of the ipsilateral lumbar spinal cord was compared with that in the blood, identifying chemokines and transcription factors as potential blood-borne biomarkers of neuropathic pain. The top-down approach

explored the utility of saccadic eye movements as an objective, functional biomarker of sedation, an adverse effect associated with opioid treatment of pain. This study compared the interaction between sleep deprivation and opioids on opioid-naïve with opioid-tolerant participants. The naive-participant study evaluated the effects of sleep deprivation alone, morphine alone and the combination; the tolerant-participant study compared day-to-day effects of alternate-daily-dosed buprenorphine and the combination of buprenorphine on the dosing day with sleep deprivation. Psychomotor impairment was measured using saccadic eye movements, other oculomotor measures and an alertness visual analogue scale (VAS). Saccadic eye movements demonstrated an additive interaction between acute opioids and sleep deprivation, however the nature of the interaction between chronic buprenorphine and sleep deprivation remained unclear. This study revealed greater saccadic eye movement, but not VAS impairment in tolerant versus naive participants, suggesting that chronically dosed patients may not become tolerant to the sedative effects of opioids. These findings open up a number of new opportunities for pain biomarker development within the peripheral immune system, identify potential pain biomarker candidates, as well as further validating saccadic eye movement analysis as a biomarker of sedation. This thesis highlights that bottom-up and top-down approaches are appropriate methods for biomarker discovery and development.

## Declaration

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Grace PM, Hutchinson MR, Manavis J, Somogyi AA and Rolan PE. A novel animal model of graded neuropathic pain: Utility to investigate mechanisms of population heterogeneity (2010) Journal of Neuroscience Methods 193 (1): 47-53.

Grace PM, Stanford T, Gentgall M and Rolan PE (2010) Utility of saccadic eye movement analysis as an objective biomarker to detect the sedative interaction between opioids and sleep deprivation in opioid-naive and opioid-tolerant populations. Journal of Psychopharmacology. Epub Feb 8

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Peter Michael Grace 1 November 2010

## Statement of Authorship

Grace PM, Hutchinson MR, Manavis J, Somogyi AA and Rolan PE. A novel animal model of graded neuropathic pain: Utility to investigate mechanisms of population heterogeneity (2010) Journal of Neuroscience Methods 193 (1): 47-53.

Impact Factor: 2.295

Mr. Grace had a major input in the experimental design, performed most surgeries, most behavioural testing, tissue collection immunohistochemistry imaging and densitometry, statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

Signed 1 November 2010

Dr. Hutchinson was involved in the experimental design, performed some surgeries and conducted some behavioural testing, assisted with tissue collection, contributed to the data interpretation and preparation of the manuscript.

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Mr. Manavis conducted the immunohistochemistry and contributed to the preparation of the manuscript, including writing much of the immunohistochemistry section of the Methods.

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Prof. Somogyi was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Grace PM, Hutchinson MR, Bishop A, Somogyi AA, Mayrhofer G and Rolan PE (2010) Adoptive transfer of peripheral immune cells potentiates allodynia in a graded chronic constriction injury model of neuropathic pain. Brain Behavior and Immunity (*in review*).

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Mr. Grace had a major input in the experimental design, conducted all experimental procedures except intrathecal injections, statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

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Dr. Hutchinson was involved in the experimental design, performed intrathecal injections, assisted with tissue collection, contributed to the data interpretation and preparation of the manuscript.

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Mr. Bishop assisted with the flow cytometry and contributed to the preparation of the manuscript.

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Prof. Somogyi was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Assoc. Prof. Mayrhofer was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Grace PM, Somogyi AA, Rolan PE and Hutchinson MR. Potential biomarkers of pain identified by RNA microarray correlation of ipsilateral dorsal horn of the lumbar spinal cord with whole blood in a graded chronic constriction injury model of neuropathic pain. *Text in manuscript*.

Mr. Grace had major input in the experimental design, performed surgeries, behavioural testing and RNA purification, statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

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Prof. Somogyi was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Dr. Hutchinson was involved in the experimental design, contributed to the data interpretation, assisted with statistical analyses and preparation of the manuscript.

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Mr. Grace had a major input in the experimental design, recruited all participants, conducted all experimental procedures, some statistical analysis and all graphical presentation of the data collected, and prepared the manuscript for submission.

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Mr. Stanford performed the majority of statistical analyses and contributed to the preparation of the manuscript, including writing much of the statistics section of the Methods.

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Mrs. Gentgall was responsible for the clinical trial logistics and provided editorial assistance.

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Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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## Abbreviations

| | |
|---|---|
| 5-HT | 5-hydroxytryptamine/ serotonin |
| AMPA | $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid |
| ASIC | Acid-sensing Ion Channel |
| ATP | Adenosine triphosphate |
| AVAS | Alertness visual analogue scale |
| BDNF | Brain derived neurotrophic factor |
| BK | Bradykinin |
| BOLD | Blood oxygenation level dependent |
| CB | Cannabinoid |
| CCI | Chronic constriction injury |
| CGRP | Calcitonin gene related peptide |
| CIP | Compact integrated pupillograph |
| CNS | Central nervous system |
| COX | Cyclooxygenase |
| CSF | Cerebrospinal fluid |
| CSGAAS | Cardiff saccades generating and analysis system |
| DA | Dark Agouti |
| DLF | Dorsolateral funiculus |
| DRG | Dorsal root ganglion |
| DSST | Digit symbol substitution test |
| EBN | Excitatory burst neuron |
| ECF | Extracellular fluid |
| EOG | Electro-oculography |

| | |
|---|---|
| EW | Edinger Westphal |
| FEF | Frontal eye field |
| fMRI | Functional magnetic resonance imaging |
| GABA | $\gamma$-aminobutyric acid |
| GFAP | Glial fibrillary acidic protein |
| GKO | Gene knockout |
| IASP | International Association for the Study of Pain |
| IBN | Inhibitory burst neuron |
| IFN | Interferon |
| IL | Interleukin |
| IN | Internuclear neuron |
| i.p. | Intraperitoneal |
| i.t. | Intrathecal |
| LC | Locus coeruleus |
| LDI | Laser Doppler imaging |
| LIP | Lateral intraparietal area |
| LPS | Lipopolysaccharide |
| MAPK | Mitogen activated protein kinase |
| medRF | Medullary reticular formation |
| MHC | Major histocompatibilty complex |
| MVN | Medial vestibular nuclei |
| N | Neuronal |
| NA | Noradrenaline |
| NF$\kappa$B | Nuclear factor $\kappa$B |

| | |
|---|---|
| NGF | Nerve growth factor |
| NMDA | N-methyl-D-aspartate |
| NNT | Number needed to treat |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| NPH | Nuclei prepositus hypoglossi |
| NRS | Normal rat serum |
| OIH | Opioid induced hyperalgesia |
| OPN | Omnidirectional pause neurons |
| PAG | Periaqueductal grey |
| PAMP | Pathogen-associated molecular patterns |
| PAT | Pupil adaptation test |
| PBMC | Peripheral blood mononuclear cell |
| PET | Positron emission tomography |
| PG | Prostaglandin |
| PLR | Pupil light reflex |
| PNL | Partial nerve ligation |
| PNS | Peripheral nervous system |
| PO | Postoperative |
| PPRF | Paramedian pontine reticular formation |
| PSV | Peak saccadic velocity |
| QST | Quantitative sensory testing |
| ra | Receptor antagonist |
| rCBF | Regional cerebral blood flow |

| | |
|---|---|
| ROS | Reactive oxygen species |
| RPD | Resting pupil diameter |
| RVM | Rostral ventromedial medulla |
| S | Subcutaneous |
| S1, 2 | Somatosensory cortex, primary, secondary |
| SC | Superior colliculus |
| SD | Sprague Dawley |
| SEF | Supplementary eye fields |
| SEMs | Saccadic eye movements |
| SG | Substantia gelatinosa |
| SNL | Spinal nerve ligation |
| SSRI | Selective serotonin reuptake inhibitors |
| TASK | Tandem of P domains in a Weak Inward rectifying $K^+$ channel-related acid-sensitive $K^+$ |
| TCA | Tricyclic antidepressant |
| $T_H$ | Helper T cell |
| TLR | Toll like receptor |
| TNF | Tumour necrosis factor |
| TREK | Tandem of P domains in a Weak Inward rectifying $K^+$ channel -related $K^+$ channel |
| trkA | Tyrosine kinase receptor A |
| TRPA | Transient receptor potential subfamily A |
| TRPV | Transient receptor potential vanilloid |
| VAS | Visual analogue scale |