Systematic review and meta-analysis of studies using animal models of neuropathic pain protocol

Neuropathic pain is defined as a "pain caused by a lesion or disease of the somatosensory system" [1] and can result from diverse causes including diabetes, spinal cord injury and nerve trauma. Pain cannot be directly measured in non-human animals; however, many models of diseases or drug treatments known to induce neuropathic pain in humans have been developed. Such models are used to investigate the pathophysiology underlying the disease and to test novel therapies. For ease of reference these models will be referred to as ‘models of neuropathic pain’. These diverse models of neuropathic pain aim to recapitulate the myriad of causes, and the outcome measures used to assess hypersensitivity and efficacy of therapies vary significantly.

The primary aim of this project is to use a systematic approach to establish a large dataset of experiments using models of neuropathic pain. These will include in different neuropathic pain paradigms and use different pharmacological treatments. We will generate empirical evidence to refine experimental design and to reduce the number of animals used.

In this review the following animal models will be included;

1. **drug-induced**
   - (anti-retroviral drug-induced, chemotherapeutic agent-induced, acrylamide-induced, chymopapain-induced and 5,7-dihydroxytryptamine (5,7-DHT)-induced)

2. **disease-induced**
   - (herpes zoster-induced, human immunodeficiency virus (HIV)-induced, diabetes-induced; ethanol-induced; ethanol withdrawal-induced; hypoglycemia; vitamin B12 deficiency; syphilitic myelitis; epidural abscesses with spinal cord compression; degeneration of the spinal cord due to vitamin B12 deficiency; dysraphism; syringomyelia)

3. **peripheral nerve trauma models**
   a. physical trauma to the nerve (axonotomy/transection, crush, ligation, constriction, compression, electrode, ischemia (as a result of compression)) or root/brachial plexus (rhizotomy, avulsion, constriction) or DRG (compression)
   b. non-physical injury to the nerve (radio frequency ablation, freezing, photochemical, pronase poisoning, Lysophosphatidylcholine (LPC), Lysophosphatidic acid (LPA), laser & erythrosin, ischemia (as a result of occlusion))

Where **peripheral nerves** include: sciatic nerve and its branches, median nerve, ulnar nerve, spinal nerve, infraorbital nerve saphenous nerve and **roots** include dorsal root and ventral roots.

4. **spinal cord injury**
   - (compression, contusion, transection and hemisection, dorsal column injury; corticospinal tract injury, photochemically-induced ischemia; intraspinal injection of the AMPA/metabotropic receptor agonist quisqualic acid (QUIS)) or intraspinal injection of N-methyl-D-aspartate (NMDA))
Only models which are primarily neuropathic pain models with injury or disease of the somatosensory system will be included. We will exclude the following:

cancer pain (e.g. cancer-induced bone pain and pancreatic cancer); osteoarthritis; fibromyalgia; cerebral abscesses; complex regional pain syndrome (e.g. ischemia/reperfusion injury on the hind limb using a tight fitting O-ring); non-compressive lumbar herniated intervertebral disc in which the DRG is exposed and transplanted with nucleus pulposus; DRG is exposed and transplanted with nucleus pulposus following incision of the epineurium; pancreatitis; colitis; cervical facet joint distraction (whiplash); post-operative pain (e.g. plantar incision); colorectal distension-induced visceral pain; kidney disease; tuberculosis; opioid-induced models (e.g. opioid-induced hyperalgesia, morphine withdrawal hyperalgesia, nociceptin-induced, dynorphin-induced); intraplantar injection of nerve growth factor; cyclophosphamide cystitis; allodynia and hyperalgesia evoked by intrathecal admin of Noc/OFQ; transient trigeminal ganglion ischemia; formalin; glycine antagonist strychnine; Complete Freund Adjuvant (CFA); sciatic inflammatory neuropathy (induced by perisciatric injection of zymosan); migraine headache; pyridoxine-induced neuropathy; zymosan-evoked; bilateral painful neuropathy induced by the unilateral antigen application to the sciatic nerve; venom-induced models (e.g. cobra venom); resiniferatoxin-induced pain; neuropathy induced by feeding animals with clioquinol; neuropathy induced by dichloroacetate exposure; ovarietomy-induced hyperalgesia; diabetes + CFA/formalin/fenvalerate; central pain syndrome induced by application of benzylpenicillin on the spinal cord; PGE\textsubscript{2}-induced neuropathy; lipopolysaccharides (LPS)-induced neuropathy; organophosphorus-induced delayed neuropathy (OPIDN) (e.g. induced by tri-ortho-cresyl phosphate (TOCP)); equine laminitis pain; models of multiple sclerosis (including Experimental Autoimmune Encephalomyelitis (EAE)); post-stroke central pain; postthoracotomy pain (fourth and fifth intercostal nerve ligation); experimental autoimmune neuritis (EAN) (an animal model of acute inflammatory demyelinating polyradiculoneuropathy (AIDP) that is the most common subtype of Guillain-Barre syndrome (GBS)); Charcot-Marie-Tooth disease type 1A and Controlled Cortical Impact.

Objectives

1) to establish whether a test with a high burden of pain or distress might be replaced with one of lower impact
2) to establish whether multiple tests are necessary
3) to establish whether it is possible to identify tests which, because of the variance observed, require fewer animals
4) to establish whether the duration of experimentation may be shortened by comparing data reported from earlier and later time points
5) to assess the impact of post-operative analgesia on outcome
6) to assess the impact of internal and external validity of studies on reported outcome
7) to look for the presence of publication bias and identify the magnitude of this problem

Inclusion Criteria

- **Types of studies** – whole animal models of neuropathic pain where behavioural outcomes are presented.
- **Types of animals** - whole animals excluding humans.
- **Characterisation** - include studies which characterise animal models of neuropathic pain.
- **Pharmacological intervention** - include studies which test a pharmacological intervention
- **Transgenic studies modelling neuropathic pain** - include studies which use transgenic animals to model a disease associated with neuropathic pain e.g. diabetes.
- **Types of outcome measured:**
  - Behavioural outcomes including (but not an exhaustive list) evoked behavioural outcomes (e.g. von Frey, Plantar Test (Hargreave's Method) and thermal footplate); spontaneous behavioural outcomes (e.g. weight bearing difference, spontaneous foot lifting and burrowing); motor tests (e.g. rotarod); CatWalk method and complex behavioural outcomes (e.g. elevated plus maze, open field and conditioned place preference). Where graphs compare a pain model versus control we will extract the time point at which the behavioural change is most severe (i.e. the time point at which there is the largest difference between model and control). Where graphs compare a neuropathic pain model + treatment versus neuropathic pain model + vehicle we will extract the time point at which the drug is most effective (i.e. the time point at which there is the largest difference between treatment and control).
  - Animal welfare measures such as autotomy score
  - Anatomical outcomes
  - Electrophysiological outcomes (e.g. measures of peripheral or central activity)
  - Neurochemical outcomes (e.g. glial cell markers, c-Fos, CGRP and substance P)
- **Controlled studies** - outcomes must be compared to a suitable control where the same animal cannot be used for both e.g. contralateral is not a suitable control for ipsilateral.

**Excluding**
- Publications or abstracts without data
- Multiple models presented as a single model e.g. HIV plus anti-retroviral drug.
- Transgenic studies investigating the pathophysiology of neuropathic pain e.g. knock out animals which receive surgery to induce chronic constriction injury.
- In vitro studies
- Genomic studies (expression arrays etc)
- Case reports
- Any human studies
- Letters and comments
- Retrospective veterinary studies
- Studies where treatment is given before neuropathic pain is induced (e.g. treatment given before CCI surgery).
  - Outcomes where co-treatments are given (e.g. Gabapentin + Tramadol). We are only extracting data where animals have been given a monotherapy.

**Search strategy for identification of studies**
We will carry out a systematic search of 5 online databases (PubMed, Web of Science, Biosis Citation Index, Biosis Previews and Ovid Embase) to identify all in vivo studies of animal models of neuropathic pain. The following search terms will be used:
(neuropathic pain OR neuropathic OR allodynia OR neuralgic) OR ((neuropathy) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((shingles OR postherpetic OR herpes zoster OR varicella zoster OR herpetic OR herpes simplex virus) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((HIV OR human immunodeficiency virus) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((d4T OR antiretroviral OR anti-retroviral) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((streptozotocin OR diabetes OR diabetic) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((chemotherapy OR paclitaxel OR oxaliplatin OR taxol) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ("nerve transection" OR "transection of nerve") AND (pain OR analgesia OR analgesic OR alldonyia OR neuralgia)) OR ("contusion injury OR hemisection OR dorsal column injury OR corticospinal tract injury OR complete transection OR spinal cord injury) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((nerve compression) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((nerve injury) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ((nerve ligation) AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR (("chronic constriction injury" OR "chronic constrictive injury") AND (pain OR analgesia OR analgesic OR alldonyia OR neuralgia)) OR ("nerve ligation") AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ("nerve compression") AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ("nerve injury") AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ("nerve ligation") AND (pain OR analgesia OR analgesic OR allodynia OR neuralgia)) OR ("chronic constriction injury") OR ("chronic constrictive injury") AND (pain OR analgesia OR analgesic OR alldonyia OR neuralgia))

Limit: other animals.

Additionally, we will attempt to include unpublished data reporting the use of gabapentin, in the first instance, in models of neuropathic pain from pharmaceutical companies.

The publications will be screened to remove duplicates, reviews, case reports and retrospective studies. Reviews will be kept in a separate folder to be check for additional studies. The included publications will be divided according to the type of neuropathic pain model being studied e.g. chemotherapeutic agent-induced, sciatic nerve chronic constriction injury, herpes zoster-induced etc and data will be extracted from publications one model at a time. If a publication includes more than one model type the data will be entered and this will be recorded in a field in the database.

Methods of the review

Risk of bias assessment – Study quality will be assessed against a checklist comprising (i) publication in peer-reviewed journal, (ii) randomisation to treatment or control, (iii) allocation concealment; (iv) blinded assessment of outcome, (v) statement of sample size calculation, (vi) statement of compliance with animal welfare regulations, and (vii) statement regarding possible conflicts of interest. Studies will be awarded one point for each item they report. We will also measure the quality of these domains via the modified form of the Jadad quality scoring tool [2].

The modified Jadad scoring tool will award a point for those studies which report (i) randomisation, (ii) blinding of animal allocation, (iii) blinding of assessment of outcome, (iv) a description of dropouts, and (v) a description of a power calculation. Additional points are given if the methods for randomisation (vi) blinding of animal allocation (vii) and blinding of assessment of outcome (viii) are described and an additional point if the (viii) methods and assumptions for a power calculation are described. Points will be deducted if the methods of randomisation or blinding are inappropriate or unclear or an inappropriate power calculation is described. Additionally, we will extract data on the animal welfare
regulations that publications reported following i.e. institutional guidelines or International Association for the Study of Pain (IASP) guidelines or both.

**Data Extraction** – For a study to be included a behavioural assessment must have been carried out in the experiment. From each source we will identify individual comparisons where outcome is measured in a group of neuropathic pain animals and compared with outcome in a control group at specified times. Control groups will be defined as; animals that have not received the surgery or agent used to induce pain, for characterisation experiments; animals that have not received the drug treatment, for drug studies and wild-type animals, for transgenic studies. For each comparison reported we will extract data for number per group, mean outcome and its standard deviation. Where data are only given graphically in publications we will estimate values by measurement from graphs using Universal Desktop Ruler software. We will also collect other relevant data including the time of outcome measurement and the animal model of neuropathic pain used, as well as the individual terms of the methodological quality assessment checklist detailed below. We will extract specific details on the neuropathic pain model including duration of model and latest time point measured.

From each source we will identify:

1) **Characteristics of neuropathic pain model**: species, strain, sex, age, weight, injury induction method, site of induction, anaesthetic used during surgery and type of sham model used.

2) **Characteristics of experiment**: animal husbandry and outcome assessment details.

3) **Timings**: time after induction of injury to sacrifice plus time interval between induction of injury and outcome assessment.

4) **The outcomes assessed**: Behavioural outcomes: e.g. evoked responses (e.g. von Frey thresholds, and response to thermal footplate), spontaneous responses (e.g. weight bearing difference between hind limbs and spontaneous foot lifting duration), motor responses (e.g. latency to fall from the rotarod) and complex responses (e.g. exploratory behaviour on the elevated plus maze or open field and time spent in compartments in conditioned place preference), **Histological outcomes in the brain, spinal cord, dorsal root ganglia, peripheral nerves and skin**: e.g. expression of markers of; glial cells, C-fos, p38alpha/beta, substance P, NMDA receptor subunits and CB1/CB2 receptors. **Electrophysiological outcomes**: e.g. activity in dorsal horn neurons and size of receptive field.

5) **In studies using histology, number and size of sections, stains used.**

6) **If n numbers are given as a range** the most conservative estimate will be extracted, e.g. n=6-12 entered n=6.

**Analysis** – Data will be aggregated using weighted mean difference random effects meta-analysis performed within the CAMARADES analysis package using Microsoft Access. Where a single control group serves multiple treatment groups, the size of the control group entered to the meta-analysis will be adjusted by division by the number of treatment groups served. Stratified meta-analysis will be performed according to therapeutic drug dose; time of administration; study quality; components of study quality checklist; presence of co-treatments; type of animal model; species and gender of animal used; outcome measure used; and interval to quantification of outcome. We plan to analyse rats versus mice separately.
Meta-regression techniques will be used to compare findings from studies where different outcomes have been measured in the same cohort of animals. This will provide evidence to establish whether less noxious tests are as predictive as more severe alternatives and whether a test with a high burden of pain or distress might be replaced with one of lower impact. Additionally, such analyses will allow us to establish whether multiple tests are necessary. Secondary analyses will be performed to provide precise estimates of the variance of different outcome measures allowing robust sample size calculations to be performed and reported. This analysis will establish whether it is possible to identify tests which, because of the variance observed, require fewer animals. Meta-regression techniques will be used to compare the data reported at earlier time points with those at later time points to refine the duration of experiments. Similarly, we will compare various post-operative analgesia regimens in animal models to determine if it is possible to use robust pain relief and only cease this during the window of outcome assessment. To look for the presence of, and quantify the magnitude of, publication bias in the neuropathic pain literature using trim-and-fill analyses.

As the unpublished data that we get from Industry will not be the full dataset in addition to the conventional publication bias analysis that we perform, we will perform "trim and fill" analysis on just the subset of data provided by industry to look at "declaration bias".

**Protocol Amendments January 2014**

**Models to exclude**
- Cerebral abscesses; kidney disease; tuberculosis; venom-induced models (e.g. cobra venom); resiniferotoxin-induced pain; neuropathy induced by feeding animals with clioquinol; neuropathy induced by dichloroacetate exposure; ovarietomy-induced hyperalgesia; diabetes + CFA/formalin/fenvalerate; central pain syndrome induced by application of benzylpenicillin on the spinal cord; PGE2-induced neuropathy; lipopolysaccharides (LPS)-induced neuropathy; organophosphorus-induced delayed neuropathy (OPIDN) (e.g. induced by tri-ortho-cresyl phosphate (TOCP)); equine laminitis pain; models of multiple sclerosis (including Experimental Autoimmune Encephalomyelitis (EAE)); post-stroke central pain; postthoracotomy pain (fourth and fifth intercostal nerve ligation); experimental autoimmune neuritis (EAN) (an animal model of acute inflammatory demyelinating polyradiculoneuropathy (AIDP) that is the most common subtype of Guillain-Barre syndrome (GBS)); Charcot-Marie Tooth disease type 1A and Controlled Cortical Impact.

**Models to include**
- Ethanol withdrawal-induced; hypoglycemia; Lysophosphatidic acid (LPA) injury to the nerve.

**Additional inclusion criteria**
- Behavioural data of pain-related outcomes must be presented.

**Additional exclusion criteria**
- Publications or abstracts without data.
- Where multiple models are presented as a single model e.g. HIV combined with anti-retroviral.
- Studies where treatment is given before neuropathic pain is induced (e.g. treatment given before CCI surgery).
- Outcomes where co-treatments are given (e.g. Gabapentin + Tramadol). We are only extracting data where animals have been given a monotherapy.
Data extraction

- From graphs comparing neuropathic pain model versus control we will extract the time point at which the behavioural/sensory change is most severe (i.e. the time point at which there is the largest difference between model and control).

- For graphs comparing neuropathic pain model + treatment versus neuropathic pain model + vehicle we will extract the time point at which the drug is most effective (i.e. the time point at which there is the largest difference between treatment and control).

- Autotomy score will not be classed as a pain-related outcome measure but will be recorded as an animal welfare measure.

- We will extract specific details on the neuropathic pain model including duration of model and latest time point measured.