



What can rats tell us about neuropathic pain? Critical evaluation of behavioral tests used in rodent pain models

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Abstract

Background and Purpose: Animal models are a necessity in the study of neuropathic pain, and much of what we know about pain comes from studies in mice and rats. However, very few basic discoveries have been translated so far from rodent models into effective pain therapy. This review presents the most important rat models used in basic pain research, discusses their limitations and recommends better use of these models in future studies.

Materials and Methods: A critical review of existing literature on rat models of neuropathic pain is made. Different models are compared and their contribution to pain research presented.

Results: There are numerous models of neuropathic pain, and each has its advantages and disadvantages. However, much methodological diversity exists and different interpretations of behavioral tests are used to assess pain in animal models. These methodological differences need to be resolved in order to achieve translation of research results into successful clinical therapeutics.

Conclusions: Results of behavioral tests in animal pain models should be considered with caution. Further refinement of animal models of pain and associated methodologies is important. More work is needed for determination of the most predictive animal models, removal of user bias and introduction of more complex outcome measures in behavioral tests.

Pain research became a hot topic in the 1970s and 1980s when publication of the gate control theory and the founding of the International Association for the Study of Pain (IASP) attracted many researchers to the field and sparked an explosion in pain research. Much of what we know about pain comes from studies in mice and rats, and the goal of pain research has always been development of practical applications that can lead to pain relief for those who need it.

Patients have the right to be pain free. However, their physicians still rely on the »old« drugs, initially developed for indications other than neuropathic pain, a special pain condition that results from damage to or dysfunction of the nervous system. Meta-analyses of randomized clinical trials (RCTs) have identified several drugs that are effective across a range of neuropathic pain disorders (1). The majority are tricyclic antidepressants, opioids, topical sodium channel blockers and anti-epileptic drugs with diverse mechanisms of action. Most of them

have been shown to be efficacious in animal models of neuropathic pain, perhaps demonstrating the utility of animal models for detecting »true positives« (2,3).

Unfortunately, beside the lack of new drugs, there are still remarkable gaps in our understanding of the topic. Very few recent basic research discoveries have been translated from animal pain models into effective pain therapy. In this review we will explain why this is so and what is in our opinion the main obstacle in basic pain research using rodent models. We believe that these issues need resolution before anyone can indulge in the relatively more direct and gratifying studies of channels, neuropeptides or kinases.

Animal models in pain research

A common limitation in development of a certain research field is the lack of the usable animal model. In pain research this problem is even more pronounced due to the subjective nature of painful experience. Only humans have the ability to express and describe the emotional aspect of a painful experience. However, there are numerous problems with the use of patients or healthy volunteers in pain research. We can only use modest stimulus that will not produce any irreversible harm and we also have to take into account accompanying diseases, malingering and placebo effect. It is also very difficult to recruit significant numbers of patients needed for clinical trials. Therefore, pain research is often conducted using animal models. Examination of the pathogenesis of neuropathic pain has been accelerated by the introduction of rodent models of nerve injury that produce behavior indicative of spontaneous and inducible pain. Such models try to mimic human disease and result in measurable and reproducible sensory deficits (allodynia, hyperalgesia, and spontaneous pain) over a sustained period (4) (for terminology see (5)).

Numerous animal models reflecting human neuropathic pain syndromes have been developed and characterized. These models generally involve some kind of mechanical injury to the peripheral nerve, although the method used to induce injury varies. Animals with these types of nerve injury have been shown to develop abnormal pain sensations similar to those reported by neuropathic pain patients. Only a few models will be mentioned here. The first and simplest of the models was described by Wall *et al.* and involved complete transection of the sciatic nerve at midhigh level (Figure 1) (6). This resulted in a completely anesthetic limb and self-amputation (autotomy), which was used to quantify the degree of neuropathic pain (6). Other models involve partial injury, which preserves a subset of afferent fibers and results in altered sensory function. The first widely used model of incomplete peripheral nerve injury was chronic constriction injury (CCI, Figure 1), in which four ligatures of chromic gut suture produce partial axotomy (transection of the neuronal axon), ischemia, or inflammation (7). The spinal nerve ligation (SNL) is a model in which the fifth and sixth lumbar (L5 and L6) spinal nerve components of the sciatic nerve are ligated close to their respec-

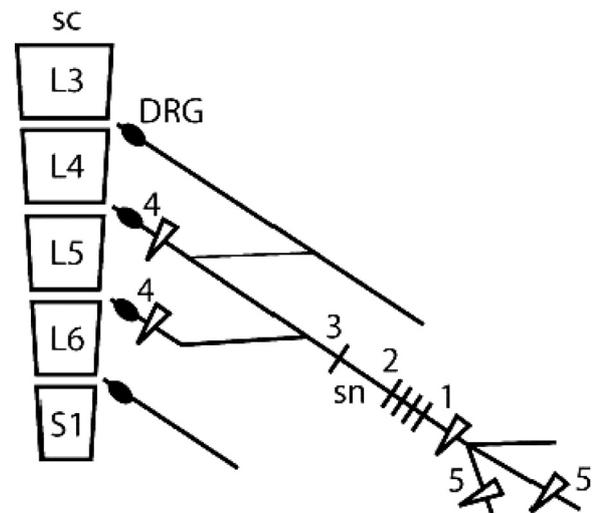


Figure 1. Schematic representation of the anatomical location of the nerve injury in different rat pain models: 1) complete sciatic transection (CST) (6); 2) chronic constriction injury (CCI); 3) partial sciatic nerve ligation (9); 4) spinal nerve ligation (SNL) (8); 5) tibial and sural transection (TST) (10). Legend: sc – spinal cord, sn – sciatic nerve, DRG – dorsal root ganglia.

tive ganglia producing spontaneous pain, mechanical and heat-evoked hyperalgesia lasting at least 4 months (Figure 1) (8). Seltzer *et al.* developed the partial sciatic nerve ligation model by tightly ligating 1/3 to 1/2 of the sciatic nerve with a single ligature (Figure 1) (9). Recently Lee *et al.* transected different combinations of the three branches of sciatic nerve (tibial, sural and common peroneal nerve) to investigate which combination produced the most robust and stable degree of allodynia and hyperalgesia (Figure 1) (10).

Comparisons between these models confirmed huge variability between them in the duration and degree of neuropathic pain symptoms (11, 12).

In addition, there are numerous other animal models for other types of pain such as: inflammatory pain models (formalin or carrageenan injection), musculoskeletal, visceral, postsurgical, or cancer pain models that will not be reviewed here. Also, there are some models that pharmacologically modulate sensory pathways without attempting to emulate clinical conditions (intradermal capsaicin, systemic antiganglioside antibody, or intrathecal strychnine). These models have less direct relevance to typical human diseases and are not the subject of this review (4). Since there are numerous excellent reviews on the topic of experimental animal models (4, 13) we will direct our review towards some of the problems that trouble researchers while attempting to use or understand the results of nerve injury models.

What are we measuring?

Since pain is »an unpleasant sensory and emotional experience« (5, 14), the best we can do in animal experimentation is to record behavior and infer the animal experience. Since all our tests have inherent weakness of in-

ference about animal experiences we can only detect pain-related behavior as indirect evidence of pain. Multiple studies have illustrated that the laboratory environment has a robust effect on behavioral traits through many factors: the experimenter, season/humidity, time of day, sex, testing surface, stress, housing, handling, or habituation (reviewed in (15–18)). Our research group has shown that the type of surgical procedure can also substantially influence pain behavior (19). The results of behavior testing can also depend on the tools used. The most common stimulation tools used for testing pain-related behavior are von Frey filaments that allow us to measure withdrawal from a progressive stimulus. Unfortunately, threshold testing for withdrawal from low-intensity mechanical stimulation with von Frey fibers are altered even after sham surgery without nerve section and also contralateral to the nerve section (20–23). This means that withdrawal testing with von Frey filaments does not distinguish specific ipsilateral injury effect from contralateral effect or from the general condition of the injured animal. The only way to avoid the bias of a shift in general excitability superimposed on injury effects is to test ipsilateral and contralateral paws and subtract contralateral results. There are excellent reviews dealing with pain testing methodology, some of them addressing the big problems which we are facing in behavioral testing (15, 24).

However, it would be presumptuous to conclude that we do not have reliable tests and that even the tests that we have are not used as they should be. Beside von Frey fibers numerous inventive methods for behavioral testing have been devised. Validation of the behavioral test done by our group revealed that the best method for detection of injury effect is the pin prick method (22). When a pin is applied with moderate pressure to the footpad of a rat the response is either a brief reflex withdrawal or a more complex, hyperalgesic reaction characterized by sustained lifting, shaking, and licking of the paw (22). As the latter response occurs only after true SNL but not sham exposure of the nerve alone, and only on the side ipsilateral to the injury, this may be accepted as an indication of a neuropathic pain state. In our opinion complex and sustained hyperalgesia response as an indicator of neuropathic pain is the best outcome measure. Others have also recognized that complex responses provide better ways for detecting the real effects of injury (25). Complex responses could be estimated through operant models (26) or even through facial expression of rats in pain. It was demonstrated that social communication of pain among mice tested together was visually mediated (27). This finding suggests that mice may communicate their pain through the display of facial expressions. Therefore, a standardized coding system for mice facial expressions was formulated, similar to the Facial Action Coding System (FACS) in humans (28), based on still photographs (captured from digital video) of mice facial expressions taken in the presence or absence of a tonic painful stimulus (0.9% acetic acid, i.p.). This effort resulted in the Mouse Grimace Scale (MGS), comprised of six »facial action

units,« scored on a scale of 0 (not visible) to 2 (severe), which can be used to make global pain-no pain discriminations. Preliminary results demonstrate an accuracy of approximately 75%, where most errors in signal detection are 'misses' rather than potentially more dangerous 'false alarms'. At this stage, inter-rater reliability is approximately 70%, but both accuracy and reliability percentages are expected to increase with practice and stimulus refinement, potentially including multiple angle shots per subject, increased resolution, and the addition of dynamic facial expressions captured by video. Presuming that this scale can be used for various types of pain and that accuracy improves with training and practice, the ability to reliably detect pain from facial expressions of mice may contribute significantly to pain research, providing valuable information about an animal's subjective experience that may otherwise be inaccessible (29).

Detection of neuropathic pain in a rat model of neuropathic pain

Decreased threshold for reflex withdrawal from a tactile stimulus, although commonly used as an outcome criterion has not been validated as an indicator of an unpleasant experience. In order to dissociate the specific neural injury effect of the model from nonspecific global changes in sensory responsiveness and distant non-neural injury effects (30,31) validation of this methodology has to identify patterns of change in behavior that are a) relevant to the human experience of neuropathic pain, b) that are predominantly ipsilateral to injury and c) more evident in fully injured animals than in those subjected to sham surgery.

Validation done by our group showed that there is a substantial variance in all sensory tests at baseline. After surgery, tests using brush, cold, or heat stimulation showed minimal distinctions between surgical groups. Postsurgical thresholds for withdrawal from mechanical stimulation with von Frey fibers were decreased bilaterally in SNL and sham groups. In contrast, the probability of a complex hyperalgesia-type response with prolonged elevation, shaking, or licking of the paw was selectively increased on the ipsilateral side in the SNL group. Nonetheless, the effect of SNL on behavior was inconsistent, regardless of the sensory test. The behavioral measure that best distinguishes between SNL and sham groups and thereby best identifies animals with successful SNL-induced neuropathic pain is increased ipsilateral postsurgical probability of a hyperalgesia-type response to noxious mechanical stimulation. We have shown that, despite inherent variability in sensory behavior and inconsistent expression of nerve injury effects, a subgroup of tests would distinguish those SNL animals that successfully exhibited a specific local effect representing animal neuropathic pain. What is more important is that we proved that simple withdrawal from von Frey tactile stimulation, although frequently used, is not a valid measure of peripheral nerve injury pain in rats, whereas a complex hyperalgesic-type response is specific neuropathic-induced behavior.

Species and strain differences in rodent sciatic nerve anatomy

Most of above mentioned experimental pain models were developed in rats. However, there is a strong motive for use of these models in numerous genetically characterized inbred mice strains, particularly those genetically modified. Unfortunately, the application of behavioral assays of nociception to transgenic mice has been inconsistent and sometimes of poor quality, leading in some cases to non-replicable and misleading conclusions. One of the reasons was that existing rat hindlimb pain models were simply transposed to mice. These assumed sciatic nerve neuroanatomical similarities have been recently investigated by Rigaud *et al.* (32). They found that in three strains of mice, the sciatic nerve is composed of elements originating from the L3 and L4 spinal nerves with a smaller diameter contribution from L5 and with no contribution from the L6 spinal nerve. In rats the sciatic nerve is composed predominately of elements originating at levels L4 and L5, i.e. 98-99% of the somata of primary afferent neurons projecting to the sciatic nerve resides in the L4 and L5 DRG (32,33). In rats contribution from L3 and L6 is small and variable (34).

The Rigaud study also revealed that mice with a short lumbar vertebral column showed a shift in relative contribution to the sciatic nerve by L3 and L4. Ligation of the mouse L4 produced hyperalgesia similar to that in rats after L5 ligation. We can say that L3 and L4 segments are anatomically and functionally homologous with rat L4 and L5 segments (32). Therefore the results of experimental distal hind limb inflammation, cancer, or sciatic nerve injury in mice must be sought in the L3 and L4 neuronal segments. Performance of the SNL model in mice should entail ligation of the L4 spinal nerve and possibly the L5, while axotomy-induced changes in neighboring, intact neurons will be evident in the adjacent L3 DRG (32).

The influence of segmental level on areas of nerves contributing to the sciatic nerve differs between strains. This is evident from the dissimilar proportionate contributions of L3 and L4 in the different strains. Specifically, for DBA and F2 mice, the L3 and L4 spinal nerve contribution is relatively equal, whereas in the C57 strain, the L4 contribution is greater than the L3 contribution. In comparison, the L5 spinal nerve gives a proportionately smaller contribution in all three mouse strains, and the L6 spinal nerve does not contribute at all (32).

In contrast to mice, however, the L4 and L5 spinal nerves provide the primary contribution to the sciatic nerve for all three rat strains. This confirms prior observations of the dominance of the L4 and L5 segments in forming the sciatic nerve in the rat (33,35). There was a significant interaction between strain and segmentation in areas of spinal nerves contributing to the sciatic nerve. Specifically, Wistar rats have an equal contribution by L4 and L5 spinal nerves, whereas Sprague-Dawley and Brown Norway rats exhibit a disproportionate contribution from the L5 nerve compared to the L4 nerve. The

L6 nerve gives a much smaller contribution to the sciatic nerve (only 5–10% of that of the L5 nerve), but this is highly variable, as some rats show no contribution from the L6 spinal nerve.

The smallest segmental source of the sciatic nerve was always the most caudal of the three spinal nerve origins (L5 in mice, L6 in rats). There was variability between strains, however, in the proportions provided by different levels. The dominant root of the sciatic nerve may even differ between strains, as L4 is largest in C57 and F2 mice, whereas L3 is somewhat larger than L4 in DBA mice.

In mice the analysis of the spinal bones showed substantial variability between strains with respect to the number of lumbar vertebrae. Specific comparisons between mice strains with six instead of five lumbar vertebrae showed a caudal shift in contributions to the sciatic nerve, so that L4 rather than L3 predominated. These data suggest that the presence of five *vs.* six lumbar vertebrae in mice may affect the balance of contributions by the specific spinal nerves to the sciatic nerve (32). When compared, all examined rat strains consistently had six lumbar vertebrae. However, the observed different patterns of spinal nerve contributions to the sciatic nerve in different rat strains indicate the existence of additional factors other than bony segmentation that influence relative contributions of lumbar spinal nerves to the sciatic nerve in rats (32).

Challenges of interpreting research results from rodent pain models

The findings discussed in this review raise a critical issue of how to interpret many prior studies in which withdrawal was the only criterion for detecting pain-related behavior or in which the L4/L5 dominant composition of the sciatic nerve in rats was incorrectly assumed to hold true in mice.

Several types of studies are involved. Those that examine effects of spinal nerve axotomy on DRG somata are unlikely to be influenced by incorrect level identification since tissue harvest was almost certainly performed at the same level as the axotomy. A greater problem arises in mouse studies that have investigated primary afferent somata after inflammation or injury at more peripheral sites such as the paw or sciatic nerve in the thigh. These reports have sought changes in the L4 and L5 DRGs, not L3 and L4 as indicated by Rigaud and colleagues (32).

Several considerations determine whether the different anatomy of mice should influence the interpretation of those reports. Firstly, it is possible that the mice used in those studies are different to those used in the study by Rigaud and colleagues (32). Secondly, in the case of incorrect set of ganglia the results would differ only partially because L4 DRG would correctly reflect peripheral effect. Also, there is a possibility of correctly harvested L3 and L4 and incorrect nomenclature. This may happen if the sciatic nerve is followed proximally and the proximal origins are assumed to be L4 and L5. A final setting in

which identification of lumbar nerve levels may critically influence research findings is performance of the SNL model. Published studies in mice have described pain behavior after ligation of the L5 spinal nerve, with (36,37) or without (38–40) ligation of the L6 nerve. Proof of only a minor contribution of the mouse L5 spinal nerve to the sciatic nerve predicts that L5 ligation will minimally affect the population of afferents going to the foot or sciatic nerve in the thigh (32). Potential explanations for these divergent claims are similar to those listed above. The correct ligation (L4) may have been performed with the wrong nomenclature. Alternatively, the incorrect (L5) spinal nerve may have been ligated in these studies, analogous to an exclusive ligation of L6 in rats, for which plantar sensory changes are unknown but probably minor.

On the other hand these strain differences in sciatic nerve composition could explain observed differences in the degree of thermal and mechanical hypersensitivity that follow L5 SNL (38). Alternatively, if the L4 nerve was correctly ligated and only the numerical nomenclature was incorrect, a genetic effect may result from the variable extent of injury due to different contributions of the L4 spinal nerve to the sciatic nerve and the variable proportion of surviving fibers in L3. Observed inter-strain differences in the proportionate composition of the sciatic nerve in rats, may in part explain dissimilar behavioral responses to SNL in different rat strains (41).

Call for uniform reporting standards

Instead of a conclusion we invite all those interested in animal pain models to use the results of behavioral tests critically and with caution. Further refinement of animal pain models and associated methodologies, is important for the pharmaceutical industry and are objectives of the European Union Innovative Medicines Initiative (http://imi.europa.eu/calls-01_en.html). Topics to be addressed in this initiative include determination of the most predictive animal models, removal of user bias from accepted models and measures of more complex behavior than simple withdrawal reflexes. The first step in that direction could be usage of an Extended Methods Form suggested by a group of authors and Preclinical Pain Consortium (Supplementary material in (3)).

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