Anthony H Dickenson * and Jane E Haley
*Neuroscience, Physiology and Pharmacology, University College, London WC1E6BT and Edinburgh Neuroscience,University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ
Corresponding author AHD anthony.dickenson@ucl.ac.uk

Brain Research 50th Anniversary Special Issue

Evidence for spinal N-methyl-d-aspartate receptor involvement in prolonged chemical nociception in the rat

Haley Jane E, Sullivan Anne F, Dickenson Anthony H.

Abstract
We used in vivo electrophysiology and a model of more persistent nociceptive inputs to monitor spinal cord neuronal activity in anaesthetized rats to reveal the pharmacology of enhanced pain signaling. The study showed that all responses were blocked by non-selective antagonism of glutamate receptors but a selective and preferential role of the N-methyl-d-aspartate (NMDA) receptor in the prolonged plastic responses was clearly seen. The work lead to many publications, initially preclinical but increasingly from patient studies, showing the importance of the NMDA receptor in central sensitisation within the spinal cord and how this could relate to persistent pain states.

Keywords
Pain; spinal cord; N-methyl-d-aspartate (NMDA) receptors; central sensitisation

Subcutaneous injection of formalin into the hindpaw peripheral receptive field of deep dorsal horn multireceptive (convergent) nociceptive neurones was used to produce a prolonged (1 h) activation of the cells. This chemical noxious stimulus produced a first peak of firing which lasted 10 min followed by a second peak of prolonged activity which was monitored for 50 min. γ-d-glutamylglycine (DGG), a non-selective N-methyl-d-aspartate (NMDA) and quisqualate/kainate (non-NMDA) receptor antagonist was applied intrathecally both as a pretreatment and after the formalin. A complete abolition of both peaks of the formalin response was produced by DGG pretreatment (1000 μg) (n = 4). This dose produced profound inhibition of the acute C-fibre evoked responses of the same cells. However, no inhibitions were produced when the antagonist was applied once the formalin response had developed (n = 4). The selective NMDA receptor antagonist 5-amino-phosphonovaleric acid (AP5) was administered intrathecally (250 and 500 μg) as a 40 min pretreatment and caused a small inhibition of the first peak but a marked dose-related reduction in the second prolonged phase (n = 7). AP5 did not influence the C-fibre inputs onto the cells. The non-competitive NMDA receptor channel blockers, ketamine and MK801, were administered i.v. during the second phase of firing. Ketamine (1-8 mg/kg) caused a short-lasting but marked and dose-related inhibition of the neuronal responses to formalin (n = 11). MK801 (0.5-1 mg/kg) resulted in a prolonged inhibition of cell firing during the second phase of the response (n = 11). When administered intravenously as a 30 min pretreatment, MK801 (0.5-1 mg/kg)

produced a dose-related inhibition of the second phase of firing with only a small inhibition of the first phase. Finally, blockade of peripheral afferent activity during the first peak by 2% lignocaine administered into the site of the formalin injection did not alter the second peak in any way (n = 10). It therefore appears that the afferent barrage produced by formalin induces NMDA mediated central activity over a relatively short time span and that once induced this activity could be one basis for changes in nociception and its modulation during longer-term pain states.

During the late 1980's the idea that pain inputs could be amplified in the periphery was well established [Woolf 1983] but the concept of amplification occurring at the level of the spinal cord was just beginning to be considered. The idea that pain wasn't all in the periphery; that it was changeable and not immutable and arose as a result of changes in the spinal cord was a fundamental shift in thinking. Such plastic changes within the central nervous system had already been demonstrated in the hippocampus; electrophysiological recordings had been used to reveal that long-term potentiation (LTP) was a model system for examining activity-driven amplification of signals and that this phenomenon was involved in spatial memory formation [see Collingridge and Bliss 1995]. From these studies we knew, therefore, that the high frequency stimulation used to induce LTP elicited enough depolarization in the post-synaptic neurone to recruit glutamate NMDA receptors and that this resulted in a long-term amplification in the pathway [see Ji et al 2003]. Furthermore, the availability of antagonists at the NMDA receptor allowed pharmacological dissection of this phenomenon. There was some evidence that glutamatergic inputs into the spinal cord were capable of activating NMDA receptors since a previous study of ours, [Dickenson and Sullivan 1987] coincident with a great paper by Davies and Lodge [Davies and Lodge 1987]], showed that low frequency stimulation produced a short duration potentiation of signals at spinal cord dorsal horn neurons (termed 'wind up'), and this was prevented by blocking the NMDA receptor.

So, in 1990 not much was known about central hypersensitivity in pain or the neuronal substrates that could be involved. In order to understand more, we used a technique that allowed us to extracellularly record the firing activity of dorsal horn neurones directly, *in vivo in the anesthetized rat*. The lab was well placed to use this technique as Tony Dickenson had learnt *in vivo* extracellular electrophysiology recording in Paris as a post-doc [Le Bars et al 1979] and had brought the technique to UCL when he established his own lab in 1983. Previously we had used this technique to examine the inhibitory controls exerted by the different opioid receptors on dorsal horn activity in response to stimulation [Dickenson and Sullivan 1987]. We now felt that it could be used to explore in more depth the excitatory inputs and, coupled with pharmacological tools, we could tease out what were the transforming events that might lead to central sensitization.

Unlike LTP, which generally requires high frequency stimulation to be elicited, we wanted to use a more natural stimulus. The subcutaneous injection of formalin into the hindpaw had previously been used as a behavioural stimulus and shown to

produce pain-related behaviour that lasted for an hour [Coderre et al 1990], which is much longer than the few seconds of stimuli generally used for algesia tests such as the tail-flick. It was hardly chronic pain but the duration of the effect allowed for pharmacological manipulations and a more persistent stimulus. The work was done by two young fantastic scientists, Jane Haley, who was doing a PhD and Ann Sullivan who joined as a technician but who also gained a PhD. The formalin response was generally very reliable and I recall that the study was finished in a short period of time. We chose Brain Research for publication since we felt that the concept was an important one and might resonate across many neuroscience domains.

So what was found?

Following a single bolus injection of formalin into the hindpaw, we saw a biphasic pattern in the activity in the dorsal horn neurons: an immediate, acute, phase of activity that rapidly faded and was then followed by a delayed but more sustained second phase and this mirrored the behavioural response. Since glutamate is the major transmitter in pain transmitting C-fibres, a non-selective antagonist of all non-NMDA receptors abolished both electrically stimulated C-fibre activity and the formalin responses. But intriguingly, it failed as a post-treatment. The theory that the second, prolonged, phase of the formalin response was due to NMDA receptor activation was confirmed when the antagonist, AP5, selectively blocked this response but was without effect on acute pain responses. This strongly suggested a role of the NMDA receptor in the hypersensitivity at the spinal level. We then further verified this by using two more NMDA channel blockers given systemically, MK-801 and ketamine and validated the previous findings. Finally, we used a rather neat manipulation – we blocked the peripheral input with local anaesthetic so preventing the first peak and showed the second persistent NMDA mediated response was unchanged.

Thus the paper showed, for the first time, that spinal hypersensitivity uses NMDA mediated events when pain becomes persistent and that this plasticity is rapidly induced.

So what happened next?

The paper was seminal in translational research on pain. The concept that spinal hyperexcitability could be mediated centrally, via the NMDA receptor, and could be modulated by an already-licensed pharmaceutical – ketamine - lead to the use of this drug in pain patients [Dickenson 1990, Woolf 2011, see Fiorelli et al 2015,]. The description neural plasticity translated to many pain patient groups and has since been shown to be a marker for persistent pain. In more general terms, the idea that pain could be enhanced by central mechanisms has been important in helping health care professionals to believe that patients can experience high levels of pain despite minor peripheral signs.

Within a few years, the central hypersensitivity theory was being used to explore novel therapies. We now know that the peripheral consequences of nerve injury and inflammation are very different and that the short term inflammatory models such as formalin are not necessarily indicative of persistent inflammation such as arthritis, for which models now exist. Although there were models of nerve injury available from the early 90s, these were hardly characterized in terms of drug effects.

However, as a result of both animal models and human data, central hyperexcitability it is now recognized that as a relatively common event in many pains of different origins [Baron et al 2013, Clauw 2015, Dickenson 1990, Woolf 2011]

Although most published studies on spinal hypersensitivity for the next decade were from academics, one key paper from the pharmaceutical industry was published in 1997 [Hunter et al 1997]. Hunter et al was a landmark paper but, very tellingly, the title was “The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain”. So, even here, the concept that acute tests may not be predictive of persistent pains is acknowledged. They further state, “This ability to acutely reverse a prominent manifestation of neuronal sensitization demonstrates the potential of these drugs as analgesics for the relief of chronic pain following tissue or nerve injury. Moreover, the negligible effect of these drugs against an acute, high threshold thermal noxious stimulus suggests a selective interaction with pathways associated with pathophysiological events rather than with normal sensory nociceptive function.” So following our study and others using the formalin test, and based on the differential effects of drugs if given before or after the start of the pain state, the concept of preemptive analgesia arose and still is a key issue in patients [see Fiorelli et al 2015].

Today, the other concepts from the paper still hold. A series of reviews on the subject by Woolf and colleagues, who also carried out a series of studies on the substrates for central sensitization, followed and a recent review covers the clinical implications of the findings on neuronal plasticity in spinal pain signaling [Baron et al 2013, Clauw 2015, Dickenson 1990, Woolf 2011] . One important point from the paper was that peripheral drives were absolutely required for the manifestation of excitability - since lidocaine blocked the neuronal responses for the duration of its action, but as the effect wore off, the central neurones regained their activity. Thus, these pain related events would seem to differ from LTP where the enhanced activity in hippocampal circuits remains for hours after the conditioning stimulus irrespective of the continuation of stimulus input [Collingridge and Bliss 1995]. This makes sense for pain processes since it is very clear that, where possible, blocking the peripheral inputs by local or regional anaesthesia can be effective therapies [Baron et al 2013].

The presence of central sensitization is a risk factor for chronic post-surgical pain in those undergoing certain surgical procedures [Petersen et al 2015] and the concept has been shown to relate to pain in many patient groups [Baron et al 2013]. For example, the spread of pain around the original site of injury and abnormal wind-up can be clearly seen in patients with osteoarthritis. Ketamine is used in patients, often during surgical procedures to dampen hyperexcitability and in patients with cancer pain. The problematic side effects of the drugs can be circumvented in the former setting where the patients are under anaesthesia but attempts to avoid interference with forebrain NMDA receptor function have not materialized so that sedation and cognitive side-effects still prevail. Promising attempts to produce NR2B blockers did not lead to drugs for patients but there is promise in other drugs that target effects

downstream of the NMDA receptor, including interference with scaffolding proteins and the production of nitric oxide in neurones, which may allow uncoupling of the pathophysiological adverse effects of the receptor function leaving more physiological functions unaltered [D'Mello et al 2011]. However, there are drugs that indirectly modulate these NMDA functions in pain. For example, the signs of central sensitization can be seen in the brain by fMRI imaging and this activity can be modulated by the drug gabapentin that interacts with spinal calcium channel functions. Consequently, the presence of central sensitization predicts the actions of the drug.

So, the intervening 25 years have seen major steps to understanding pain, and in translating basic science to the patient and improving treatments. We are proud that our paper has been part of this process.

Acknowledgements: AHD has funding from The Wellcome Trust Pain Consortium.

References:

Baron R, Hans G, Dickenson AH. **Peripheral input and its importance for central sensitization.** Ann Neurol. (2013) 74: pp630-6.

Clauw DJ**. Diagnosing and treating chronic musculoskeletal pain based on the underlying mechanism(s).** Best Pract Res Clin Rheumatol. (2015);29:pp6-19.

Coderre TJ, Vaccarino AL, Melzack R**. Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection.** Brain Res. (1990);535:pp155-8.
PMID: 2292020

Collingridge GL, Bliss TV. **Memories of NMDA receptors and LTP.**
Trends Neurosci. (1995) 2: pp54-6.

Davies SN, Lodge D. **Evidence for involvement of N-methylaspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat.** Brain Res. (1987); 424: pp402-6.

D'Mello R, Dickenson AH **Spinal cord mechanisms of pain**. Br J Anaesth. (2008); 101: pp8-16.

D'Mello R, Marchand F, Pezet S, McMahon SB, Dickenson AH. **Perturbing PSD-95 interactions with NR2B-subtype receptors attenuates spinal nociceptive plasticity and neuropathic pain.** Mol Ther. (2011); 19: pp1780-92.

Dickenson AH**. A cure for wind up: NMDA receptor antagonists as potential analgesics.** Trends Pharmacol Sci. (1990); 11: pp307-9.

Dickenson AH, **Sullivan** AF**. Electrophysiological studies on the effects of intrathecal morphine on nociceptive neurones in the rat dorsal horn.** Pain. (1986); 24: pp211-22.
PMID: 3754322

Dickenson AH, Sullivan AF. **Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation.** Neuropharmacology. 1987; 26: pp1235-8.

Fiorelli A, Mazzella A, Passavanti B, Sansone P, Chiodini P, Iannotti M, Aurilio C, Santini M, Pace MC. **Is pre-emptive administration of ketamine a significant adjunction to intravenous morphine analgesia for controlling postoperative pain? A randomized, double-blind, placebo-controlled clinical trial.** Interact Cardiovasc Thorac Surg. (2015) ;21 : pp284-90.

Hunter JC, Gogas KR, Hedley LR, Jacobson LO, Kassotakis L, Thompson J, Fontana DJ. **The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain.** Eur J Pharmacol. (**1997)**18; 324 : pp153-60.

Iannetti GD, Zambreanu L, Wise RG, Buchanan TJ, Huggins JP, Smart TS, Vennart W, Tracey I. **Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans**. Proc Natl Acad Sci U S A. (2005); 102: pp18195-200.

Ji RR, Kohno T, Moore KA, Woolf CJ. **Central sensitization and LTP: do pain and memory share similar mechanisms?** Trends Neurosci. (2003);26 : pp696-705.

Le Bars D, Dickenson AH, Besson JM**. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat.** Pain. (1979); 6 : pp283-304.

Petersen KK, Arendt-Nielsen L, Simonsen O, Wilder-Smith O, Laursen MB. **Presurgical assessment of temporal summation of pain predicts the development of chronic postoperative pain 12 months after total knee replacement.** Pain. (2015); 156: pp55-61.

Woolf CJ. **Evidence for a central component of post-injury pain hypersensitivity.** Nature. (1983); 306: pp686-8.

Woolf CJ. **Central sensitization: implications for the diagnosis and treatment of pain.** Pain. (2011); 152: ppS2-15.