Experimental behaviour testing: pain

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Behavioural testing is a widely used method in pain research in animals, particularly in rodents. With a wide range of tests available, it is easy to tailor experiments to examine specific brain or spinal cord areas, such as the dorsal horn. This editorial outlines some of the more commonly used tests and gives examples of how they can be used to further our knowledge of neurological processes in disease conditions such as neuropathic pain or to assess the efficacy of therapeutic strategies.

Pain sensation starts in receptors in the skin and viscera which project up to the spinal cord. The majority of sensory inputs to the spinal cord terminate in the dorsal horn, where afferents carrying the signal from the periphery converge onto interneurones or projection neurones. Some inhibitory or excitatory modulation can occur in the spinal cord via the interneurons before the signal continues to the brain.1 Sometimes, before the signal reaches the brain, efferent fibres from the spinal cord also project to the muscles surrounding the initial stimulation to elicit a reflex, such as withdrawal to protect the body from the noxious stimulus.2 The various pathways involved in carrying and modulating pain sensations from periphery to the brain involve complex circuitry and the majority of neurotransmitter types found in the higher centres of the central nervous system are present in the dorsal horn.3 In the spinal cord, the majority of interneurones either release glutamate or GABA (often in conjunction with glycine).4 Glutamate is an excitatory neurotransmitter, so activates the next cell in the pathway, while GABA and glycine are inhibitory, so reduce the effect of the next neurone along. Using these two neurotransmitters, incoming signals can be modulated, that is, strengthened or dampened down, before reaching the brain. The hierarchical organization of the nervous system, highlighting the sites of action of the more commonly used pain models and nociceptive tests, are illustrated in Figure 1.

Models of pain induction

Different pain tests can be classified as thermal, mechanical, or chemical and can test for periods as short as a few seconds (acute), up to a few months (chronic) depending on the stimulus used.5

Formalin injection

Formalin injection is a commonly used acute nociceptive model which elicits a two-part response; an initial peripheral pain, driven by TRPA1 receptor and C-fibre activation, and a later response involving inflammation and central sensitization of the dorsal horn.6,7

Nerve ligation

Nerve ligation is a commonly used method of chronic pain, involving surgery to tie off one of a number of spinal nerves or peripheral nerves to induce a neuropathic pain phenotype.6,10 Ligation of spinal nerves S5, often combined with ligation of S6, results in a consistent phenotype of heat hyperalgesia, and allodynia induced by cold or mechanical stimuli. Inadvertent damage to surrounding nerves will result in visible defects such as paralysis of hindlimbs for damage to S4, a simple safeguard against inadvertently ligation the incorrect nerve.9

Heat injury

Heat-induced injuries can be induced by a number of methods. Most common models involve immersion of limbs or small portions of skin to water ranging from 60°C to 100°C.11 This gives a reliable burn injury which can be studied for histological and behavioural outcomes. By varying temperature and time immersed, severity of the burn injury can be easily controlled. Other models involve injection of drugs such as capsaisin which, while not causing a burn themselves, elicit a burning sensation and hyperalgesia to heat.12 These capsaicin models have also been applied to human studies.13

Testing pain-related behaviour

The type of behavioural test used will naturally depend on the pain model being studied. For example, tests may not be useful in models where the animal remains anaesthetized throughout, and complex behavioural test involving learning and memory may be too stressful for an animal in high levels of pain, for example, after a burn injury over a large area of the body. In this

case, measures of cellular activation via c-Fos and morphological changes may be more informative.  

**Hot-plate test**

The hot-plate test involves placing an animal on a metal plate heated to ~55°C. The animal is monitored until either jumping or licking of the hindpaw is observed, at which point the animal is removed. The hot-plate test is an acute painful stimulus, thermal in nature. The tail-flick test is of a similar nature, where the tail is placed on a hot spot and latency to moving the tail is recorded. Other thermal tests involve the use of a focused light beam instead of heated plate.

**von Frey test**

The von Frey test involves the use of a thin flexible filament applied to the skin with just enough force to induce a bend in the filament. While this test is not itself painful, it can be used to measure the effectiveness of analgesics, and can be painful in a model of hyperalgesia. Owing to the simplistic methodology, the test can be widely used with little variance, and is often used in a clinical setting.

**Conditioned place preference**

Conditioned place preference is a model which, similar to many standard behavioural tests, relies on the memory of rodents. Using two chambers, both differentiated by scent and visual cues, linked by a neutral chamber, the preference of an animal to a chamber based on its association with an analgesic drug can be measured. By administering a noxious stimulus, then allowing an animal access to a chamber where they were administered either an analgesic treatment or one with a saline control, the preferential response of animals to different drugs can be easily measured.

**Translational research**

Behavioural tests are most often used to determine the effects of analgesic drugs, and are particularly useful in studies looking at the long-term effects of anaesthetics and postoperative analgesic drugs. One such study used the hot-plate and von Frey tests to...
assess the possibility of ketamine use to prevent postoperative hyperalgesia.\textsuperscript{21} It has been shown that while sufentanil is an excellent anaesthetic and analgesic, it can induce hyperalgesia in the days after administration, an effect known of opioid drugs.\textsuperscript{22–23} The work by Minville and colleagues\textsuperscript{21} showed the effect of pretreatment with ketamine on sufentanil-induced hyperalgesia. They discovered that rats did not develop hyperalgesia when pretreated with ketamine, as evidenced by an increased latency on the hot-plate test and decreased sensitivity to von Frey testing. This would suggest treatment with ketamine before surgery could prevent hyperalgesia induced by opioid overload, the efficacy of which has now been clinically tested.\textsuperscript{25}

Another use of nociceptive testing is to investigate the actions of anaesthetic and analgesic drugs in the presence of a noxious stimulus, to more closely mimic the real-world use of these drugs, such as during surgery. One such example uses the formalin injection or surgical incision of a paw in combination with nitrous oxide and isoflurane anaesthesia.\textsuperscript{26} Seven-day-old rat pups were exposed to 6 h anaesthetic exposure (70% N\textsubscript{2}O+0.75% isoflurane) with or without noxious stimuli and markers of cell death and inflammation were assessed. It was determined that the addition of either formalin or incision caused increased neuronal cell death in the cortex and spinal cord when compared with anaesthesia alone, which was accompanied by cognitive dysfunction. Conversely, a further study carried out using ketamine and the injection of complete Freund’s adjuvant, a test similar to the formalin injection,\textsuperscript{27} discovered that the addition of a noxious stimulus actually attenuated the neurotoxic effects associated with ketamine. These studies help to further the understanding of the anaesthetic drugs routinely used and in particular, determine their possible side-effects in a more clinically relevant setting.

Complex behavioural test such as conditioned place preference testing can be used to infer anti-nociceptive properties of new drugs. In one study examining the potential use of curcumin (an extract of turmeric, a natural analgesic) as a postoperative analgesic, mice were administered a hindpaw incision to model postoperative pain settings.\textsuperscript{28} Four days after incision, one group were administered morphine to induce place preference as a result of pain relief. However, the post-incision administration of curcumin abolished this place-preference learning, indicating these curcumin-treated animals were not feeling pain, hence the morphine held no learning reward. These findings were confirmed by measuring hyperalgesia in the incised paw by von Frey testing and a test similar in nature to the hot-plate test, and have been replicated in clinical studies.\textsuperscript{29} This demonstrates the flexibility of using multiple tests and the supportive use behavioural tests can have to strengthen findings.

**Further developments**

The use of animal models can lead to the discovery of mechanisms, side-effects, and potential treatments which could alter the clinical outlook we currently have. The tests widely used in animal research are, by and large, quite closely related to human conditions, but researchers need to be sure the tests they use and outcomes they measure are carefully planned to reflect clinical scenarios. More importantly, it is necessary to keep up to date on new findings and techniques. Langford and colleagues\textsuperscript{30} recently developed a test to rate pain in rodents using facial expression. This technique was inspired by something already used in humans and can predict pain to a high level of accuracy. In a further study, this mouse grimace scale (MGS), which measures five different facial features to assess pain, was used to test the efficacy of four commonly used postoperative analgesics for rodents.\textsuperscript{31} They discovered that buprenorphine is effective at current dosing suggestions, carprofen and ketoprofen were ineffective at the doses currently recommended for postoperative analgesia in rodents, and acetaminophen was unable to reduce pain expressions at any dose. These are worrying results as the drugs tested are some of the most commonly used postoperative analgesics in in vivo studies, so if they are ineffective, it may cause unnecessary discomfort and slower recovery in animals. This study also highlights the need for experimenters to constantly re-evaluate how they use animals in the laboratory, bearing in mind the refinement of models and techniques to improve the quality of animal care and results.

**Authors’ contributions**

S.S. and D.M.: concept and writing of manuscript.

**Declaration of interest**

D.M. is a board member of BJA.

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**References**

Anaesthetists are familiar with stress during their work, particularly in the face of an unexpected clinical emergency. As a profession, we pride ourselves in our ability to prevent and mitigate these events. We train for critical situations by reading clinical guidelines, using algorithms, and performing mental and physical rehearsals. Increasingly, the management of common emergencies is mandated in vocational training and continuing professional development to ensure one’s knowledge, skills, and preparedness to act.

Simulation training has been shown to be particularly effective in improving the ability of clinicians to manage emergency situations. Its effectiveness in improving the technical and non-technical skills required is believed to be due to the deeper engagement of the learner. In simulation-based education the learner has to be more engaged, as they must perform as they would in the actual clinical setting. The simulation learner performs all the same tasks, considers how the same resources would be best allocated, and must communicate effectively with other team members to coordinate their efforts. It has also been suggested that immersive simulation may also improve performance compared with other methods of teaching by replicating the psychological stress during the actual clinical emergency.

Psychological stress is known to impair performance during emergencies in both experimental and case studies. Stress impairs many aspects of performance, including memory retrieval.