

the patient with the control over their fainting that they require. However, if time allows, a full CBT programme gives the patient the skills to see how avoidance can perpetuate a problem and helps them to develop mastery over their cognitions and their physiology.

Sadly, clinical psychology departments in general hospitals are rare. However, community psychology services are becoming more widespread and early identification of a needle phobic patient ought to be seen as an opportunity to get them treatment for their phobia before hospital treatment, as opposed to simply managing the phobia through the use of topical anaesthetic or sedation. In fact, the use of sedation in order to allow cannulation could actually be detrimental. It removes any sense of control from the patient and denies them the opportunity to learn self-management, test cognitions, and show themselves that their feared consequences do not occur once they have the skills to control their symptoms. Even relatively short time periods can produce good results with psychological therapy, and just a few weeks' notice can provide the opportunity for significant improvement and empowerment of the patient. If this is not possible, then the strategies described by Marshall and Courtman in the November Bulletin of the Royal College of Anaesthetists¹ would be effective with phobic adults and anxious children. Allowing visits to the environment, preferably with a length of stay that allows for reductions in anxiety levels and distraction, combined with desensitization to the equipment and an enhanced sense of knowledge and control will all benefit the patient, be they adult or child. Given the strong familial links in needle phobia, obviously the 'nature/nurture' debate is relevant, and if possible the presence of a non-phobic parent would benefit a child in these circumstances. Visible parental anxiety can significantly increase children's distress in the presence of

needles, whereas an adult using humour or non-procedural talk can help children cope.⁸ Given the long-term consequences of conditioned needle phobia, all healthcare professionals have a role to play in ensuring the best possible conditions, and preventing that anxious child becoming a needle phobic adult who may refuse life-saving treatment in the future.

Declaration of interest

None declared.

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EDITORIAL III

Animal behaviour testing: memory

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Animals are often used in biomedical research for testing novel treatments, investigating currently used drugs or mechanistic studies while, with the Replacement, Refinement, and Reduction of animals in research in mind, the number of animals being used is declining. In order to obtain clinically relevant outcomes, it is sometimes necessary to investigate the histological, physiological, and behavioural outcomes of any tested

interventions in animals. When using behavioural tests in animals, learning and memory are the most commonly used subjective endpoints as there are a wide range of paradigms available which examine a variety of brain regions. It is well established that the hippocampus is one of the most important brain structures involved in learning in both humans and animals,^{1 2} and lesions in this area cause impairment in

learning, with location and severity of lesion influencing the severity of the effect on learning.^{3, 4} But it has been proven that even in the absence of the hippocampus; learning can still occur.⁵ This suggests other brain areas also play a role in the formation of new memories. Another area which seems to be important is the amygdala⁶ which has been seen to undergo long-term potentiation (LTP)-like changes during behavioural testing. Unlike the hippocampus, however, the amygdala is known to be involved with emotional responses, and appears to specifically deal with emotionally driven learning and memory in humans and rodents.^{7, 8} Thus, when testing the amygdala, it is better to use a fear conditioning challenge⁹ as the emotional aspect combined with the cognitive aspect will enhance the target area. There are also many different brain regions involved at a more local level with learning and memory, including the visual cortex, primary sensory cortex, and auditory cortex.¹⁰
¹¹ To train and test each of these areas, it is necessary to use a well-designed protocol.¹²

Common testing paradigms

Fear conditioning

One of the most well-known tests is fear conditioning (Fig. 1A–C), where animals learn to form associations between different stimuli, for example, a previously unheard sound (conditioned stimulus) and electric shock (unconditioned stimulus) as an aversive stimulus. It has been demonstrated that two neural circuits are involved in fear conditioning; the hippocampus which deals with the learning and memory,¹³ and the amygdala which deals with the emotional aspect, although it has been found that the amygdala also undergoes some learning during fear conditioning.^{9, 14}

Water maze

Another very commonly used test for small rodents is the Morris Water Maze (WM) task (Fig. 1D–F). The WM differs from normal mazes such as the T-maze as the water provides a semi-aversive stimulus which motivates the animal to seek the solution as quickly as possible. It is very often used in animal models of Alzheimer's and stroke. One useful aspect about many of the memory tests is that the initial training and the recall tests can be separated by days or even a few weeks, due to long-term memory formation, allowing for the administration of a treatment or the recovery from a surgery before testing begins. This facilitates the comparison of results of a single animal from before and after treatment to better understand the effects of an intervention, and to reduce the number of animals used.

Object recognition

A third useful test is the object recognition task, which works on the basis that rats and mice are inquisitive about new objects. The first time the animal sees an object it will investigate and spend time interacting with it, but if the same object is presented later, they should not show the same level of interest.¹⁵

Other mazes

Like the WM, the T-maze (Fig. 1G) and radial arm mazes (Fig. 1H) involve using a positive reward, such as a food pellet, as an incentive to learn and remember the layout of an area. As the name suggests, the basic T-maze is a T-shaped platform with raised walls to prevent the animal jumping out. Animals are placed at the bottom of the T, and trained to alternate between left and right arms to search for the pellet. An animal taking the same arm multiple times suggests the animal cannot remember where it has previously been, an indication of a memory deficit. The radial arm maze consists of eight arms all at the same angle to each other, with a pellet at the end of each. The animal is placed in the centre, and then the number of arms it enters is counted until all food pellets have been retrieved. The number of times an animal re-enters any arm is used as an index of memory deficit.

Translational research

These subtle memory tests have been utilized to test the side-effects of a range of anaesthetics to further understand their long-term effects. Culley and colleagues¹⁶ used the radial arm maze to test performance of both adult and aged animals exposed to nitrous oxide and isoflurane combined. The study was continued for 21 days post-exposure to track long-term effects and they discovered that in both adult and aged animals, there was a persistent decrease in ability in the radial arm maze for the weeks after a single 2 h isoflurane–N₂O exposure.

Fear condition has been successfully used to assess xenon as a neuroprotective agent against postoperative cognitive decline (POCD).¹⁷ The animals were trained in a fear conditioning paradigm the day before surgery, which consisted of fracturing the tibia while anaesthetized with either isoflurane, xenon, or a combination of xenon pretreatment and isoflurane during surgery. They found that 1 day post-surgery, xenon pretreated mice performed significantly better in memory recall tests than mice anaesthetized with isoflurane alone. The anti-inflammatory and neuroprotective properties of atorvastatin in attenuating POCD occurrence after surgery was also tested in combination with WM and fear conditioning tests.¹⁸

A comprehensive study on the toxic effects of sevoflurane¹⁹ revealed that a single 6 h exposure of 3% sevoflurane in neonatal mice resulted in deficits in both fear conditioning and social interaction testing. For both contextual and auditory-based learning, sevoflurane-exposed pups showed significant deficits at both 7 and 13 weeks after exposure. This was linked with increased cell death in various brain areas including the hippocampus and thalamus. Pups exposed to sevoflurane anaesthesia also performed poorly in social interaction tests, where the time spent interacting with an inanimate object was no different from time spent investigating a social target (i.e. another mouse), which is unusual and opposite compared with control animals who spent significantly more time with social targets. This experiment shows the ease in which various behavioural tests can be combined to reveal subtle changes in animal behaviour.

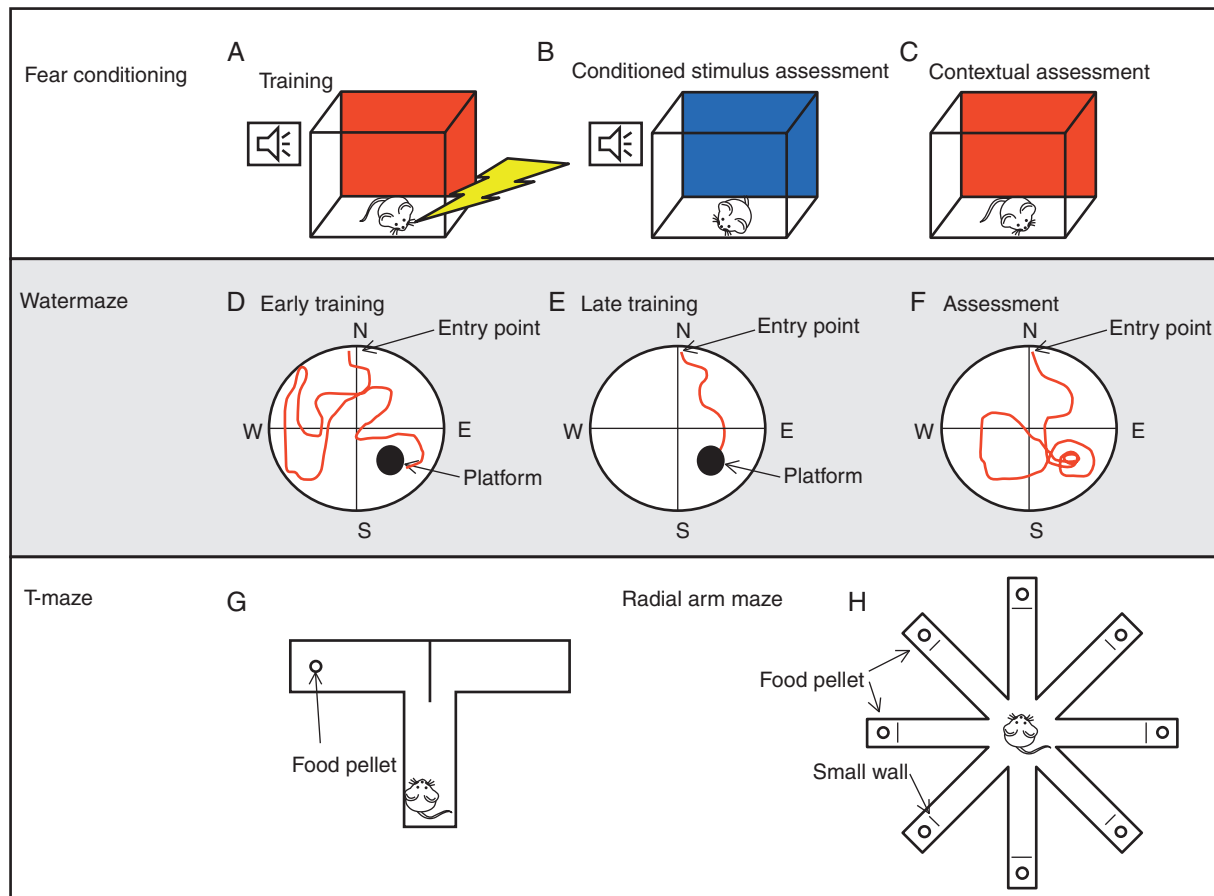


Fig 1 Basic behavioural testing paradigms. Fear conditioning: (A) shows the training setup for fear conditioning, where a conditioned stimulus (CS), for example, sound is paired with an unconditioned stimulus (US), for example, foot shock. The two forms of assessment focus on testing recognition of the CS alone (B), or the context alone (C). WM: in the most basic form of WM, training is spread over a number of days. In the early days of learning, the animal has not yet learned the visual clues, so takes some time to locate the platform (D), while in later days, they can locate the platform with little searching (E). On probe day, the platform is removed and if learning has been successful, the animal should focus searching where the platform was located during training (F). T-maze: one set-up for T-maze involves alternating a food pellet to determine if the animal correctly chooses the right arm to find the food (G). In a spontaneous alteration test, no food reward is used. Radial arm maze: (H) the animal is placed in the central area and left until each pellet has been located or time has run out. The number of entries into each arm is counted, where the number of incorrect entries (i.e. reentering the same arm or missing an arm) is used as a measure of memory. The food pellets are hidden behind small raised walls to prevent the animal seeing where it has already searched.

Further developments

The range of animal experiments incorporating behavioural tests is immense, particularly in anaesthesia research. This is due to the understanding that the neuronal manifestations of treatment mean very little unless they can be linked to changes in behaviour and performance. As can be seen from the short list of experiments above, anaesthetics can trigger not only neuro-apoptosis but can lead to further behavioural deficits either directly after a procedure or in later life after a neonatal insult.

The limitations, however, are the translation of these results into changes for clinical practice. Clinical decisions based on animal research are obviously questionable, but behavioural testing helps to extend the evidence beyond simple morphological changes. It could also help focus the symptoms clinicians should look for if clinical investigations are carried out.

For example, in one study,¹⁹ deficits were found in social interaction resembling autism-like behaviours in mice after anaesthetic exposure. This idea was also investigated by clinicians²⁰ who found similar results in children. This exemplifies the synergistic potential of using animal studies to inform clinical research with more evidence than just physiological changes and cell death. By extending these methods to non-human primates and utilizing more complex behavioural tests,²¹ basic science research comes closer to clinical relevance.

Authors' contributions

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

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Declaration of interest

D.M. (the senior author) is a member of BJA Editorial Board.

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EDITORIAL IV

Increased kidney donation rates in the Scottish NHS: a historical problem being successfully addressed

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On April 11, 2013, NHS Blood and Transplant (NHSBT) announced a ‘ground breaking 50% increase in deceased

organ donors’.¹ The UK had reached the target set after the Organ Donation Task Force (ODTF) 2008 report² and this