Functional magnetic resonance imaging (FMRI) enables the identification of the location, pattern, and time course of brain activity, in vivo, without the need for the administration of exogenous contrast agents or radioactive tracers. As FMRI can identify shared neuronal networks across species (e.g. rats, humans), it can facilitate translational research.

Owing to its convenience and relative simplicity, FMRI has become a standard tool in neuroscientific research, as it is possible to obtain objective measures of sensory, motor, and cognitive processes occurring in the human brain. The potential for FMRI in anaesthetic-related research has only recently become apparent, and anaesthetic journals are now publishing ever-increasing numbers of articles using FMRI.

The purpose of this article is to introduce the fundamentals of FMRI, to assist the non-expert reader in interpreting research articles using this technique, and to clarify issues in methodology that can confuse the unwary. In the right hands, FMRI is a powerful tool to understand human brain function in vivo. As explained below, research questions need to be chosen carefully as the technique has important limitations.

One of the main challenges in anaesthesia-related research is that altered systemic and cerebrovascular physiology may profoundly interfere with the blood flow-derived image contrast in FMRI. Furthermore, FMRI has received some criticism as a technique for being indirect, non-quantitative, and open to ‘flexible’ analysis, particularly relating to statistical methods and interpretation of findings.

How does FMRI detect brain activity?

Brain activity in the form of neurotransmitter release, uptake and cycling, and maintenance of transmembranous potential differences requires energy and is associated with a localized haemodynamic response (Figs 1 and 2). This haemodynamic response is the basis of image contrast in FMRI. The haemodynamic response consists of an increase in cerebral blood flow (CBF), cerebral blood volume, and cerebral capillary and venous oxygen saturation (because the increase in CBF leads to a greater supply of oxygenated blood to the tissue than required for the increased metabolic needs). As deoxyhaemoglobin is more disruptive to the uniformity of the magnetic field within the MRI scanner than oxyhaemoglobin, the increased venous oxygen saturation results in an increase in the MR signal, and thus image intensity, in the region of neural activation. This is known as the blood oxygenation level-dependent (BOLD) response.

Neurovascular coupling

One particular challenge with many anaesthesia-related research questions, commonly focused on a pharmacological, respiratory, or disease-related change of brain state, is their propensity to alter neurovascular coupling. Neurovascular coupling is the cascade of events that increases capillary blood flow in concert with neuronal activity (Fig. 1), and its potential for alteration must be considered when interpreting FMRI data. The following factors are particularly important to consider for their potential to alter the measured BOLD signal:

- Alterations in arterial carbon dioxide and pH have profound effects on vascular tone.
- Alterations in arterial oxygen tension can affect the oxygen saturation level of venous blood.
- Large changes in arterial pressure (i.e. beyond the limits of cerebral autoregulation, as seen in brain-injured patients) are likely to affect the CBF response to altered neural activity.
- Drugs1 or disease states2 may directly affect chemical signalling mechanisms to the cerebral vessels, vascular reactivity, cerebral metabolic rate, or physiological fluctuations and thus confound FMRI measurements.

Strategies to account for the above include meticulous control of end-tidal gases,3 or inclusion of control tasks to help understand how BOLD signal responsiveness has changed.4 Combination of FMRI with electrophysiological measurement techniques such as EEG can provide a more direct signal of neuronal activity allowing the investigation of altered neurovascular coupling.

FMRI experimental design

A typical FMRI experiment is usually conducted in a group of 12–20 subjects. Experiments involve repeatedly acquiring images sensitized to BOLD signal, alternating ‘on’ periods of
stimulation with ‘off’ periods of rest or a control condition, commonly known as a ‘block’ design (Fig. 2). For example, a painful stimulus may be applied repeatedly over a 15–30 min scanning session, and during the analysis, the painful ‘on’ periods would be contrasted with the pain-free ‘off’ periods. More sophisticated experimental designs might then explore how an intervention alters brain activity in the regions identified.6

An important consideration when designing FMRI studies is that they are best suited to an alternating ‘on’–‘off’ design within an experimental session, with stimulus blocks of up to about 1 min, and equivalent duration rest periods between. Longer or slower changes are much harder to detect, as baseline signal drift will interfere with the time course of the experimental stimulus. On the other hand, event-related designs (ultra short stimuli of a few seconds or less) are becoming increasingly popular for investigating complex brain functions, as different stimuli can be mixed. However, event-related designs have poorer statistical power and require longer experiments and are more complex to analyse.

Another important consideration in designing FMRI studies is whether alterations in physiology or motion artifacts might correlate in time with the stimulus of interest (termed ‘physiological noise’). This has recently been highlighted in FMRI studies of autism, where a popular theory of altered brain connectivity was drawn into question when it was realized that head motion could fully account for the findings.7 In this case, the autistic participants moved their heads much more than the control subjects, introducing an artifactual difference in FMRI results. Differences in head motion might, for example, confound studies of anaesthesia (when compared with awake controls) or respiratory challenges in the FMRI environment.

### Statistical analysis of FMRI data

Typically, FMRI analysis is based upon multiple linear regressions between the alternating stimuli of interest, such as depicted in Figure 2, and the signal change in each voxel (image volume element) in the FMRI time series. The FMRI scans from each subject are usually merged so that an averaged group result can be obtained. This merging involves a number of steps including registration (morphing individual brains to a common template) and blurring (smoothing) the images to account for small inter-individual differences in brain structure. During the analysis stage, correction for head motion and physiological noise5 is frequently performed.
FMRI generates large data sets comprising time courses of hundreds of thousands of voxels. These are compared mathematically with the time course of a stimulus or behavioural task to generate maps identifying brain activity as being present in voxels exceeding a statistical threshold. One important consideration is the statistical correction for multiple comparisons, necessary because of the high probability of detecting ‘false positive’ activation by chance alone somewhere in the brain when thousands of statistical tests are performed. Signal change (or ‘activity’) in neighbouring voxels is likely to be spatially and temporally correlated, because of the fact that areas of activation usually spread over multiple voxels. The Bonferroni correction treats all voxels as independent, and therefore is generally considered too stringent. Cluster-based thresholding exploits correlated signal change in neighbouring voxels, and thus ‘significance’ depends on how signal change in that voxel relates to that in neighbouring voxels. A novel refinement of this approach is to incorporate threshold-free FMRI analysis,9 which overcomes some of the limitations imposed by the choice of cluster threshold.

It is worth paying attention to the statistical methods used in any FMRI study to avoid over-interpreting data presented. Two common techniques of which to be aware of are small volume corrections and indirect comparisons. With small volume corrections, analysis is performed within a limited brain region, reducing the number of multiple comparisons, inflating statistics. Although this approach is appropriate when testing specific a priori hypothesis about activity in a particular brain region, it is not appropriate for the more common exploratory studies aimed at identifying location and pattern of brain activity implicitly across the whole brain. Indirect comparisons are when an effect (e.g. change in brain activity relating to a drug) is observed in one population but not in another (e.g. no change in brain activity in the control group), but the two groups are not formally statistically compared. A direct statistical comparison between groups must be performed in order to make a conclusion about the presence of a difference between groups.

Statistical analysis is the subject of much discussion,10 11 as publication bias and both false-positive and false-negative findings can artificially skew the literature, and are deemed to be common considering the small sample size of many neuroimaging studies. Calls for study registries (similar to clinical trials) and more standardized approaches to the design and reporting of FMRI studies12 are gaining momentum.

**Interpretation of FMRI results**

As FMRI is a correlative technique, inferring causality and mechanisms is challenging. The best practice is to interpret FMRI findings based on simultaneously collected behavioural and physiological data using a well-controlled intervention where possible. When this is not possible, or findings are not explained adequately, comparison of activation maps with those from

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**Fig 2** Basic principles of FMRI. In its simplest form, an alternating stimulus (e.g. a flashing chequerboard alternating with rest periods for 15 s) causes alternating ‘on’ and ‘off’ periods of electrical activity in certain brain regions. This electrical activity leads to a haemodynamic response. Multiple FMRI scans (each taking about 3 s) capture the ‘on’ and ‘off’ periods. FMRI analysis regresses change in T2* in each voxel (image element) against the time course of the alternating visual stimulus, and creates a statistical map of where changes in T2* correlate with the stimulus. This is interpreted as brain activity.
Editorial

Ultra-high field FMRI

These days most FMRI studies are conducted at 3 T, whereas a decade ago 1.5 T was the norm. The key advantage of moving to higher fields for FMRI is the increased sensitivity offered to detect changes in brain activity. Currently, 7 T FMRI is in its infancy, and the associated technical challenges of increased physiological noise, motion sensitivity, and signal distortions are being addressed. However, 7 T MRI scanners are becoming more commonplace, and soon the potential for even more detailed higher resolution brain imaging will be fully realized, bringing benefits to anaesthetic research in the detailed study of brainstem and cerebrovascular function.

Why may FMRI be useful for anaesthesia research?

FMRI is an established tool for pain research, shows promise in drug development, and more recently has been applied to the understanding of anaesthetic mechanisms, brain injury, and respiratory control.

Pain

FMRI has been very successful in elucidating the basic mechanisms underlying pain in humans. In this respect, FMRI is unparalleled for studying cognitive, complex emotional processes such as pain that cannot easily be measured in animal models.

Drug development

FMRI can help understand mechanisms of drug action in the brain and thus can help the pharmaceutical industry decide whether it is worth investing in a particular compound for further development, that is, to help decision-making regarding whether a full-scale clinical trial might be worthwhile.

Anaesthetic mechanisms

Although progress has been made on how anaesthetics work at a cellular level, only more recently (due to more sophisticated MRI analysis techniques) has a more systems-based approach been used to identify where and how in the brain anaesthetics work. In human volunteers, FMRI has revealed how propofol disrupts thalamic regulatory systems, and their communication with higher cortical areas.

Brain injury

Positron emission tomography (PET) has been used for many years to better understand CBF regulation after brain injury. FMRI complements PET by safely allowing serial measures (no radiation dose) and can give information on network effects, that is, how injury in one part of the brain may affect function in another.

Respiratory control

Neural control of breathing is amenable to study with FMRI. The technique promises better understanding of opioid-induced respiratory depression, breathlessness, and potentially...
brain mechanisms pertinent to weaning from mechanical ventilation (e.g. in intensive care).

**Conclusion**

FMRI offers a non-invasive, safe opportunity to better understand human brain function, both neuronal and vascular, in vivo. Although anaesthesia-related studies present challenges in experimental design and interpretation, particularly related to physiological effects on the blood-flow-related contrast, the last few years have seen a much improved understanding of how to deal with these challenges. FMRI has now become sufficiently mature to earn its place as a useful tool for answering novel research questions posed by the anaesthetic community.

In the hands of clinical researchers, FMRI has been largely the preserve of psychologists, psychiatrists, and neurologists. However, the role of the brain in controlling consciousness and life-supporting processes including respiratory, metabolic and cardiovascular functions means that FMRI offers an exciting opportunity for anaesthesia-related research.

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