OPINION

Using human brain lesions to infer function: a relic from a past era in the fMRI age?

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Abstract | Recent technological advances, such as functional imaging techniques, allow neuroscientists to measure and localize brain activity in healthy individuals. These techniques avoid many of the limitations of the traditional method for inferring brain function, which relies on examining patients with brain lesions. This has fueled the zeitgeist that the classical lesion method is an inferior and perhaps obsolescent technique. However, although the lesion method has important weaknesses, we argue that it complements the newer activation methods (and their weaknesses). Furthermore, recent developments can address many of the criticisms of the lesion method. Patients with brain lesions provide a unique window into brain function, and this approach will fill an important niche in future research.

In 1861, Paul Broca boldly suggested that the third convolution of the inferior frontal gyrus is involved with speech production. Support for this claim came from the brain of a patient who had been able to produce only one syllable (tan), in the form of stereotyped recurrent utterances: tan-tan-tan-tan…[1]. Broca brought the brain of this patient to the meeting, allowing others to inspect the damaged region (FIG. 1). Although Broca was not the first to relate language to the left hemisphere, his finding launched a new age of discovery. Specifically, Broca's approach to localizing human brain function by studying the correlation between a behavioural disorder and the location of brain injury founded a long tradition of neuropsychological research, which has greatly advanced our understanding of brain function.

The human 'lesion method' was seminal for our understanding of functions as diverse as language, memory, hemispheric specialization, emotion, vision and motor control. For example, in the late nineteenth century, Carl Wernicke discovered that damage to the left posterior temporal cortex leads to difficulty in language comprehension[2]. Work that started in the 1950s identified the role of the medial temporal lobes in encoding long-term memories[3]. Beginning in the 1960s, Sperry's work with split-brain patients revealed that the left hemisphere has superior language and arithmetic skills, whereas the right hemisphere has better spatial skills[4]. More recent neuropsychological research has refined our understanding of how emotions are processed, with damage to the amygdala resulting in difficulty in recognizing whether faces are expressing fear[5], and damage to the left insula and basal ganglia leading to a selective difficulty in identifying disgust[6]. Work involving patients with brain damage has also shown that the posterior ventral cortex (which includes the fusiform gyrus) is involved in recognizing objects, and that the posterior dorsal regions are involved in integrating visual information with goal-directed motor responses (such as grasping a door handle)[7].

Despite the enormous contribution that the lesion method has made to our understanding of the human brain, it is worthwhile to consider whether newer imaging techniques have superseded this approach and rendered the lesion method obsolete. New methods (BOX 1) such as functional neuroimaging now enable us to measure brain activation directly in healthy subjects, avoiding many of the difficulties that are associated with inferring brain function from studies of individuals with brain damage.

In this article, we compare the traditional lesion method with modern techniques such as functional imaging. We argue that the combination of both approaches will give us new insights into the anatomical foundations of human brain function, and that lesion studies fill a unique niche in our quest to understand the intact brain. There are legitimate reasons to be cautious about the lesion method, so we review and propose emerging techniques that can be adapted to improve lesions studies and to minimize some of the limitations that have previously hampered this technique.

Correlating brain damage and behaviour

To relate behavioural function to anatomy using the lesion approach, it is necessary to identify the location and extent of a brain injury. At the time when Broca conducted his work (in the 1860s), researchers had to wait for a patient to die before they could examine the brain. Modern imaging techniques such as computerized (axial) tomography (CT or CAT scans) and magnetic resonance imaging (MRI) enable us to identify damaged brain regions *in vivo*. Moreover, these techniques have aided scientists in conducting group studies, in which it is possible to identify regions that are commonly damaged in different individuals with the same deficit.

Group studies are important because most individual brain injuries involve many functional modules of the brain, making it difficult to precisely identify the region that is required for a particular function on the basis of the behaviour of a single patient. Furthermore, single patients might not represent the general population. However, group studies also present challenges. As individual brains differ in brain shape, size and structure[8], brains from different individuals need to be transformed to a standard stereotaxic space to make group comparisons. In the 'template overlay' technique championed by Knight and colleagues[9], among others, a group of patients is selected, all of whom show a specific disorder. The lesions, as observed by CT or MRI, are transferred onto schematic template drawings[10–12] using anatomical landmarks. The aim of the technique is to superimpose the individual lesions in an overlay plot and therefore to find the location that is commonly lesioned among patients that show the same disorder. FIGURE 2 illustrates two early studies using the lesion method (but without using a control group with brain damage for data analysis).

Limitations of the lesion method

There are several fundamental problems with the lesion method that have caused neuroscientists to become critical of the technique. First, lesion studies (but not only lesion studies) assume that discrete anatomical modules deal with different cognitive functions. This assumption is often referred to as the 'modularity' or 'localization' assumption. Unfortunately, many brain functions might be carried out in a distributed manner, with large portions of the brain working in a plastic fashion rather than each region having a fixed function[13]. For example, there is evidence that neurons in the prefrontal cortex of primates can adapt their behaviour on the basis of task demands[14,15]. The modularity assumption presents a dilemma for most tools that are used

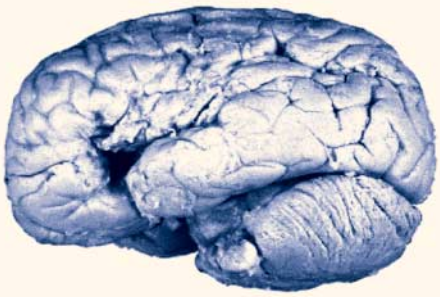

Figure 1 | **The left hemisphere of Broca's famous patient Leborgne.** This patient was only able to speak the syllable 'tan'. After Leborgne died, Broca investigated his brain and inferred that the damaged area in the left ventral frontal lobe (Brodmann's areas 44 and 45) is crucial for speech production. This pioneering work launched the lesion method in neuropsychology. Later inspection of Leborgne's brain by computerized tomography revealed that the injury included other regions of the left hemisphere[63].

today to understand brain function — regardless of whether we look at patients with brain damage or brain activity in healthy individuals.

However, the problem of modularity is particularly acute for the lesion method: even if a particular task such as speech is largely controlled by modular regions of the brain, most brain damage is not limited by the boundaries of the underlying functional modules. The location and extent of strokes are typically constrained by the brain's blood supply. For example, the middle cerebral artery supplies blood to the frontal, temporal, parietal and occipital cortices. Disruption of this blood supply usually leads to a wide range of deficits, knocking out neighbouring regions that often have different functions[16]. On the other hand, owing to the redundancy of the human brain, small lesions that only partially damage a module might not lead to any obvious behavioural problems.

Furthermore, superimposing individual lesions to identify the crucial area for a certain function assumes that these functional modules are in the same locations in different individuals. However, the brain shows great anatomical differences between individuals[8] and also shows plasticity, with different regions changing their function in response to damage to one area[17]. The lesion method usually assumes that after a focal lesion, the intact regions of the brain continue to function in the same manner as before the lesion. However, with tasks controlled by distributed and plastic circuits, the brain can start to reconfigure rapidly following damage. This reconfiguration is helpful for recovery, but makes it difficult to infer the original function of the healthy brain.

The lesion method also faces the challenge of differential vulnerability: some areas of the cortex are particularly likely to be damaged by stroke[18]. Therefore, the locations of brain damage are not randomly distributed in the brain: the design of the brain, its blood supply and the surrounding skull mean that some regions of the brain are damaged more often than others. This makes it difficult to interpret lesion overlay plots. For example, are the regions highlighted in FIG. 2 specifically involved with aphasia and with spatial neglect, or are they simply commonly damaged zones?

Brain regions can be disabled but intact after injury. Regions that appear to be structurally healthy might have their function impaired by disconnection or damage to distant brain regions that are required for earlier stages of information processing. With structural CT and MRI, it is impossible to tell whether a morphologically intact region is functioning normally. An example of this problem is illustrated by the profound cerebral dysfunction that is seen acutely after brain injury: owing to disrupted perfusion, patients who have relatively small lesions, as assessed by anatomical CT or MRI scans, can have temporary malfunctions in large regions of their brains. This presents a drawback for the lesion method: if we test patients in the acute stage of their illness, we will not be able to accurately identify all of the brain regions that are impaired. However, if we wait for these initial problems to resolve, the problems associated with brain plasticity will become more pronounced.

Finally, to understand how different regions of the healthy brain function, we need to have a good idea of the temporal sequence of information processing. This timing information is crucial for determining both the stages of processing and the roles of feedback. Unfortunately, the lesion method does not allow us to assess the time course of information processing, as most injuries are permanent. Therefore, the temporal resolution of the lesion method is exceptionally poor.

The rise of new methods

The criticisms raised in the previous section have been valid since Broca's original studies. However, in the past there were no other options for studying the function of the human brain. The advent of functional imaging markedly changed this situation, first with single-photon emission computed tomography (SPECT) and positron emission tomography (PET), and later using functional MRI (fMRI). The rise of these new methods has fuelled the current zeitgeist that the lesion method is obsolescent. Indeed, these techniques do overcome several of the longstanding methodological caveats that are inherent to the lesion method, but these new technologies also have limitations.

fMRI has huge potential and has already led to many new insights into human brain function. The fundamental idea of neuroimaging is tantalizingly simple: if we are interested in finding out which area of the brain is involved in language, we ask a healthy person to lie in a scanner whilst whispering and listening to someone speaking at certain times, and remaining silent at others. After the session, we identify which regions of the brain received more bloodflow after periods of speech and listening to speech than during silent periods. Although this description

Box 1 | Methods for inferring human brain function

Historically, examining patients with brain injury was one of the only methods by which the function of parts of the human brain could be inferred. Today, neuroscientists can choose from a broad range of techniques, each with its own strengths and weaknesses. Techniques can be compared with regards to spatial resolution, temporal precision, mode (whether the technique measures activation or interferes with the region's function) and cost. Methods that measure brain activation include electrophysiology and functional imaging. Electrophysiological tools such as event related potential (ERP) measurement and magnetoencephalography (MEG) offer excellent temporal resolution (millisecond level) but have poor spatial precision (it is difficult to infer the sources of the signals). On the other hand, neuroimaging tools such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and single photon emission computerized tomography (SPECT) offer good spatial resolution (within a few millimetres), but typically measure changes that occur over seconds. Popular brain interference methods include the 'lesion method' and transcranial magnetic stimulation (TMS). TMS aims to determine whether a region is required for a task and when the region is required. However, the neurophysiological effects of TMS are not fully understood, leading to difficulties in interpretating results[58–60]. TMS can have excitatory as well as inhibitory effects on brain regions. Differences in TMS stimulation parameters can influence the results. Moreover, present TMS systems are only able to directly disrupt regions near the scalp, usually evoke only slight changes in behaviour and can induce epileptic seizures if applied at high frequencies and intensities.

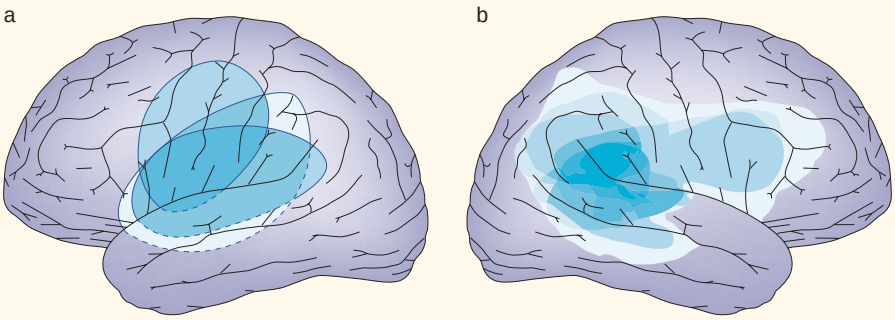

Figure 2 | **Overlay plots.** Examples of overlay plots. Regions that are commonly damaged in (**a**) three patients with Broca's aphasia after left hemisphere lesion[10] and (**b**) ten patients with spatial neglect after right hemisphere lesion[64]. Note that these overlay style images fail to take advantage of the information offered by control groups with brain damage. This style of lesion overlay plot has been adopted by many textbook chapters describing the anatomical basis of language processes in the left hemisphere and spatial attention in the right hemisphere, respectively. Panel **a** modified, with permission, from REF. 10 © (1977) Radiological Society of North America; panel **b** modified, with permission, from REF. 64 © (1983) Academic Press. Anatomical image adapted, with permission, from REF. 65 © (1996) Appleton & Lange.

belies the complexity of the experimental designs and analyses that are required to effectively use fMRI, it illustrates the fundamental concept of fMRI; identifying brain regions whose blood flow patterns correspond with the participant's behaviour.

In contrast to the shortcomings of the lesion method, the benefits of fMRI are persuasive. Importantly, because we can look at the brain activity of healthy people, we can eliminate the problems of differential vulnerability, plasticity and disconnection that are associated with the lesion method. Moreover, fMRI measures changes in bloodflow over seconds, offering better temporal resolution than can be achieved by examining permanent brain injury. Other brain activation techniques, such as magnetoencephalography (MEG) and event related potential (ERP), offer even better temporal resolution, although their spatial resolution is poorer (BOX 1). Furthermore, functional imaging can show every part of a neural network that is involved in a task or a behaviour.

Despite these advantages, the interpretation of brain activation studies can be difficult and has clear limitations[19]. We can amass evidence that a particular task correlates with activation in a particular brain region. However, it is not clear whether this region is necessary to perform this task. It is even possible that some activated areas have no direct role in information processing, but are activated because of their connections to regions that are required for a task. For example, a task that is processed entirely in the left hemisphere might produce bilateral activation because of the strong homotopic neural connections between the two hemispheres. This might explain why many studies have observed bilateral (although asymmetric) activation during, for example, language tasks[20–25]. It is clear that the left and right hemispheres have different roles in language processing, with the left hemisphere having the decisive role[26], but it is hard to infer this difference from fMRI data. The following example illustrates this problem.

Although we know from many fMRI studies with healthy subjects that both hemispheres are activated during language tasks[20–25], neurosurgeons regularly resect cortical tissue from exactly those locations where such studies have reported fMRI activation in the right hemisphere without any consequences for the patients' language facilities. On the other hand, resection of the activation foci observed in the left hemisphere during the same tasks would usually lead to profound speech deficits. Obviously, the knowledge that steers the neurosurgeon's decisions does not emerge from studies using functional imaging alone but rather from a long series of studies that have documented the consequences of left (but not right) brain damage on language, the effect of cortical electrical stimulation on human speech mechanisms[27,28] and, for example, the Wada test (which transiently disables parts of, or all of, one hemisphere[29]). As these techniques measure disruption instead of activation they can reveal the structures that are required for language function — located predominantly in the left hemisphere.

This example should not dampen the present efforts to replace invasive techniques by non-invasive ones, such as fMRI[30,31] or MEG[32], to determine the dominant hemisphere for language in a particular person before surgery. It simply illustrates the difference in inference that can be drawn from disruption techniques (such as the lesion method) as opposed to measures of brain activation.

A further aspect should be mentioned. Understanding fMRI activations is difficult, but interpreting null results (regions where no change in activity is detected) is even more challenging. The fMRI technique cannot detect the possible contributions of regions that are constantly active, regardless of the task. These regions might have a crucial role in the information processing required by the task, but their bloodflow might not be significantly modulated by the task. In other words, 'the absence of evidence is not evidence of absence'. We cannot conclude that an area that does not show changes in bloodflow during a task is not involved with that task.

So, although lesion data do not provide the precision of fMRI activation foci, they can tell us which areas are necessary for controlling a cognitive function. Techniques such as fMRI (which measures brain activation) offer a weaker level of inference than the lesion method (which measures brain disruption). Modern brain activation techniques such as fMRI, PET, MEG and ERP only show that a region is involved with a task. On the other hand, brain disruption techniques such as the lesion method enable us to infer that the region is required.

The future of cognitive neuroscience

Both the lesion method and neuroimaging techniques have clear limitations. However, several of the strengths and weaknesses of these tools are complementary. The power of cognitive neuroscience comes from using convergent tools to investigate the same theoretical question and to reveal the anatomy and time course of a given brain function. The lesion method fills an important niche in the growing arsenal of tools that are available to cognitive neuroscientists.

However, it is worth considering whether new technologies can be used to optimize the lesion method. Although some of the lesion method's limitations are inherent to the technique, other weaknesses can be addressed by recent technical innovations. In particular, emerging imaging protocols and improved analysis of lesion data should improve our ability to measure the functional extent of brain damage and help us to refine our understanding of the relationship between brain anatomy and function.

The potential of new imaging protocols

CT scans are still a mainstay for clinical use: they allow easy detection of haemorrhages, patients do not need to be screened for metal implants that are not compatible with MRI scanning, and CT scans are less expensive than MRI. Furthermore, the quality of modern

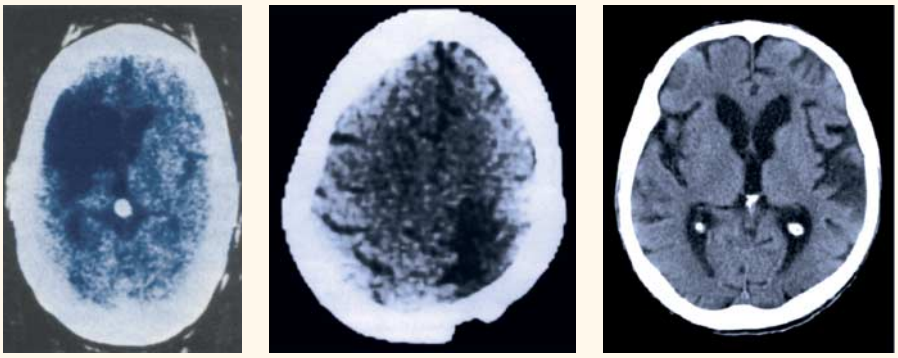

Figure 3 | **Improved computerized tomography (CT) imaging has resulted in more accurate mapping of lesion size and location. a** | CT scan from 1977 (Hayward *et al.*); although grainy by present standards, this type of image was sufficient to extend Broca's original claims and contribute to our understanding of language as described in most textbooks. This scan is an example of the images used to construct the overlay plot illustrated in FIG. 2a. **b** | Typical CT scan used in 1983 (Heilman *et al.*) to search for the regions commonly damaged in patients with spatial neglect after right hemisphere damage. This scan is an example of the images used to construct the overlay plot illustrated in FIG. 2b. **c** | Contemporary CT scan. This scan shows high resolution of tissues and anatomical landmarks, aiding scientists in identifying common regions of damage across groups of individuals with similar deficits. Panel **a** modified, with permission, from REF. 10 © (1977) Radiological Society of North America; panel **b** modified, with permission, from REF. 64 © (1983) Academic Press.

CT images offers a powerful tool for neuropsychological research. A series of recent clinical and technological innovations have created excitement because they provide clearer pictures with more detail and in less time than previous CT systems (FIG. 3).

MRI scans offer several distinct advantages over CT for neuropsychological work. In particular, CT scans measure a single property — the target's opacity to X-rays (although it is possible to change the opacity of different regions by using contrast agents such as injections of gadolinium to observe blood flow), whereas different MRI protocols can be used to emphasize different tissues and physical properties. This flexibility offers great potential for future lesion method studies.

Currently, two types of scan are most popular for MRI studies of lesions. T1-weighted scans offer good contrast between grey and white matter and have typically superior spatial precision compared with T2-weighted scans, which typically highlight regions of damage, giving good pathological information. However, both T1- and T2-weighted MRI scans have limitations. First, conventional T1 and T2 scans often fail to detect acute strokes (when clinical intervention is necessary and the pathological behaviour is severe). Second, like CT, these sequences do not necessarily show whether an area is functioning normally.

Fortunately, new protocols can overcome these drawbacks. Techniques such as diffusion-weighted imaging (DWI) enable strokes to be visualized at an early stage[33]. Although T2-weighted fluid-attenuated inversion-recovery (FLAIR) imaging sequences are sensitive to acute cerebral infarcts, DWI imaging has proved to be particularly sensitive for the detection of hyperacute infarcts and can accurately predict the final infarct size[34–37]. Furthermore, these techniques can often identify the extent of brain lesions more accurately than conventional T1- and T2-weighted images. An extension of DWI, diffusion-tensor imaging (DTI), takes advantage of the fact that water motion in the brain is constrained perpendicular to fibre tracts, but much less constrained in the direction of the fibre tracts. DTI can help to identify whether regions are disconnected after a stroke, and how the white matter has been damaged[33].

Despite the great potential of using fMRI to understand brain dysfunction, this application poses unique challenges. For example, current techniques are much more sensitive in younger people than in older people[38]. Therefore, fMRI is not a particularly sensitive tool for the typical elderly stroke patient. Furthermore, as brain injury can severely disrupt regional blood flow, fMRI might fail to detect robust activity in neurological patients for two reasons. First, brain injury can result in reduced metabolism (as the damaged region no longer absorbs nutrients), resulting in the surrounding intact areas receiving blood flow in excess of their demands (a situation referred to as 'luxury perfusion'). Second, the surviving arteries can show sustained dilation, compensating for compromised arteries. Either of these effects can reduce the task-related dilation and constriction of blood flow that is measured by fMRI as the correlate for brain activity. Consequently, a region might be functioning perfectly well, but appear to be unresponsive to standard fMRI analysis.

Techniques such as perfusion imaging can enable us to measure the amount of blood flow that reaches different regions of the brain. This is achieved by either injecting gadolinium during MRI scanning (measuring the latency and amount of blood to reach different regions), or applying arterial spin labelling to use the blood itself as a contrast agent (which can measure blood reaching the capillary beds, with little influence of blood from venous flow). For example, Hillis and colleagues[39] found that hypoperfusion of the left hemisphere was a strong predictor of aphasia, and functional recovery over time corresponded with increased perfusion. It seems that perfusion techniques can identify regions that are receiving enough energy from the blood supply to remain structurally intact, but not enough to function normally.

In conclusion, new imaging techniques will provide a better understanding of the total extent of disruption after brain injury. These tools have clear implications for improving patient care, but will also improve our theoretical understanding of brain function. Advances in imaging have been complemented by new tools for analysing lesion data; these are reviewed below.

Control patients in lesion studies

Since Broca's talk in 1861, neuropsychology has tended to focus on patients who show a disorder of interest, but to ignore those who do not show the deficit. When attempting to identify the anatomical basis of a particular behaviour by superimposing lesions in an overlay plot, scientists have often failed to recognize the value of identifying a group of control patients (see examples in FIG. 2). Simple overlay plots for patients who have a disorder can be misleading, because the regions that they highlight might reflect increased vulnerability of certain regions to injury (because of their vasculature, for example)[18], rather than any direct involvement with the disorder of interest. A control group of neurological patients who do not exhibit the deficit of interest is therefore indispensable for valid anatomical conclusions.

Broca's own work illustrates the importance of control subjects. His finding indicated that patients who have difficulties with speech production have damage to the third convolution of the frontal lobe (Broca's area). However, Broca's original findings were challenged when Poeck *et al.*[40] examined the anatomy not only of those patients who

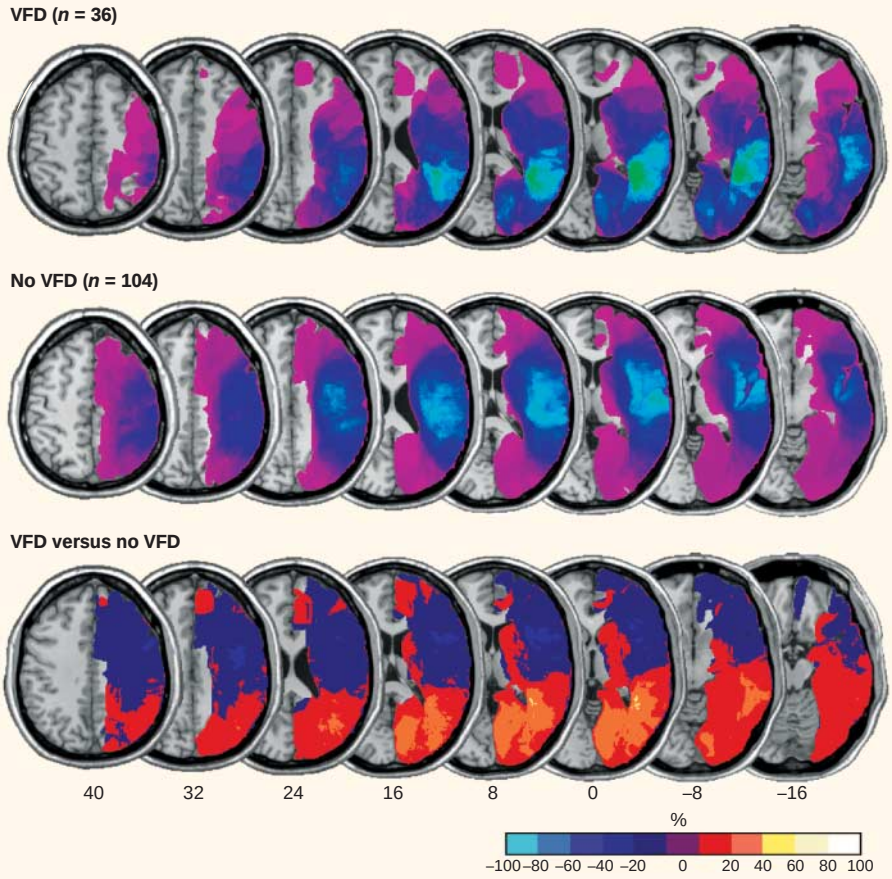

Figure 4 | **The importance of using control groups in lesion studies.** In this example, we explore the anatomy that correlates with primary visual field defects (VFD). We conducted a new analysis based on data from 140 patients with right hemisphere damage, reported in REF. 66. The top row shows a classical lesion overlay plot (similar to those in FIG. 2) for 36 consecutively admitted patients with VFD. This strategy could lead us to (erroneously) believe that primary vision is a function of subcortical white matter and the surrounding cortical region at the temporo-parietal junction. The panel in the middle shows the distribution of lesion frequency in the remaining 104 patients admitted in the same time period. They also had right hemisphere brain lesions but did not show VFD (control group). The bottom row shows a subtraction image: patients showing VFD minus controls (no VFD). The percentage of overlapping lesions after subtraction is illustrated by different colours, which code increasing frequencies, from dark red (difference = 1% to 20%) to white-yellow (difference = 81% to 100%). The colours from dark blue (difference = –1% to –20%) to light blue (difference = –81% to –100%) indicate regions damaged more frequently in control patients than in patients showing VFD. This subtraction image now accurately highlights the optic radiation and primary visual cortex as being typically damaged in patients with VFD and spared in control patients.

showed speech production difficulties but also of those who did not show such deficits. Many patients who have damage to this region do not have speech production problems[41], and damage to Broca's area is not a particularly reliable predictor of Broca's aphasia[42–44].

To show the importance of control groups for overcoming the problem of differential vulnerability owing to vasculature, consider that we are interested in identifying the regions of the brain that are responsible for speech production. If we look at a large group of patients who have problems with speech production, we will probably find that, as a group, they have damage to the frontal, temporal and parietal lobes. But does this mean that these regions are all involved with speech production? It could be that these regions are typically damaged by major strokes. Therefore, we need to compare patients who show impaired speech production with those who do not. These control patients should have brain lesions in the same hemisphere and must be similar to the patients of interest with respect to other variables, such as other neuropsychological symptoms or visual field defects. When the groups are compared, regions of the brain that are simply vulnerable to brain damage should be commonly damaged in both groups, but regions that are specifically involved with speech production should be selectively damaged in those patients who have problems in generating speech.

FIGURE 4 compares traditional overlay plots with 'lesion subtraction' displays, which can help to distinguish regions that are often damaged in strokes from regions that are specifically required for the task of interest. Subtraction analyses of lesion data contrast an experimental group of patients (a lesion overlay with positive values) with a control group (a lesion overlay with negative values)[45–50]. The resulting subtraction image specifically highlights regions that are both frequently damaged in experimental patients and typically spared in control patients. The relative incidence of damage to regions unrelated to the disorder of interest should be equally represented in both patient groups, and will therefore not be highlighted. To illustrate the importance of using control groups in lesion studies, we examine in FIG. 4 the mundane question of where primary vision is processed in the human brain by analysing patients with visual field defects after a stroke in the right hemisphere.

Statistical analysis of lesion data

Ideally, we want to know whether differences in lesion frequency (for example, between patients who show the disorder of interest and patients who do not) might be due to chance or are reliable predictors of behaviour. Four statistical methods have recently been described to address this question: voxel-based morphometry (VBM), BrainVox, voxel-based lesion-symptom mapping (VLSM) and voxel-based analysis of lesions (VAL), the latter implemented in MRIcro[51]. Each of these methods estimates the statistics on a voxel-by-voxel basis, allowing fairly high spatial precision.

A further advantage of these new tools is that they use the same spatial coordinates as are used by functional neuroimaging to describe findings from healthy subjects. This enables us to compare findings from patients with brain lesions with other fMRI and PET studies. For these techniques to work effectively, we have to align brain images from different patients into a common stereotaxic space. This 'normalization' process is a potential area for errors (BOX 2).

VBM estimates the relative grey and white matter concentrations for every voxel throughout the brain. For example, a voxel that encompasses a region of the cortex is mostly grey matter, whereas a voxel in the fibre tracts between the two hemispheres is mainly white matter. Once each brain has been segmented into grey and white matter maps, we can analyse whether different groups of people have different concentrations of these tissues. For example, patients

Box 2 | Lesion normalization: aligning brains from different individuals

Individual brains vary in their pattern of folds, size, overall shape and ventricle size. A common step for both the lesion method and for functional neuroimaging studies is to normalize or 'warp' each person's brain scan so that they all have roughly the same size, shape and orientation. Typically, we attempt to match the images from all individuals in a sample to a common template image. Providing that different research groups use the same template image, we should be able to describe similar structures, improving our ability to compare data from different studies. The quality of normalization is crucial: if different individuals' brains are not matched accurately, we will not be comparing the same regions of their brains (reducing our statistical power). Even with healthy people, normalization is a delicate process and there is no perfect solution. A system that accurately matches the locations of the major sulci will distort the volume of different regions. On the other hand, a system that rigidly preserves the overall size of brain regions will not accurately align different sulci between individuals. Some functions tend to be reliably related to specific sulcal locations, whereas the anatomy of other regions tends to be better predicted by the region's overall size. As Brett and colleagues[61] noted, even in young, neurologically healthy adults, normalization is a serious problem. Working with elderly stroke patients brings a whole new set of problems. Older stroke patients typically have larger and more variable ventricles (owing to both age-related atrophy and brain atrophy resulting from the injury). Furthermore, the injured brain region can disrupt automated normalization techniques, because the injured location will differ greatly from the template image. Brett and colleagues[62] have devised a method to tackle this: the region of the brain injury is manually identified before normalization, and during normalization this region is not used to determine the correct transforms required to realign the brain into stereotaxic space. This technique can greatly improve the accuracy of spatial normalization in individuals with brain injury. However, it offers only an approximation of brain shape, and cannot completely compensate for variability of ventricle size and other features commonly found in stroke patients. The resulting images should be manually inspected to ensure that the automated algorithm has worked correctly. In many cases, there is still no replacement for having a skilled individual manually mark the location and extent of brain injury on a high-resolution template brain image.

with temporal lobe epilepsy tend to have grey matter atrophy in the hippocampal region and in other brain regions that have strong hippocampal connections. VBM is excellent for measuring such subtle differences in grey and white matter. However, it is not suitable for patients with more profound brain damage, such as strokes, where the automated routines cannot identify that a tissue has been damaged.

BrainVox, VLSM and VAL are used when the region of brain injury is clearly defined. The lesion is manually identified for each patient (marking the damaged region), and statistical maps are generated at the group level to reveal patterns of damage. BrainVox[52] pioneered a range of new features, including digital templates for lesion overlay, integrated statistics and an ability to show brain injury maps on the rendered surface of the patient's own MRI scan. Of particular interest, BrainVox's MAP-3-NP module allows statistical comparison of lesion location with performance on neuropsychological tasks (for instance, each individual's *Z*-score performance on a language task). VLSM works on similar principles. For example, Bates and colleagues[53] used VLSM to identify , in a group of patients with different degrees of language impairments, those regions responsible for deficits of speech production and for deficits of speech perception.

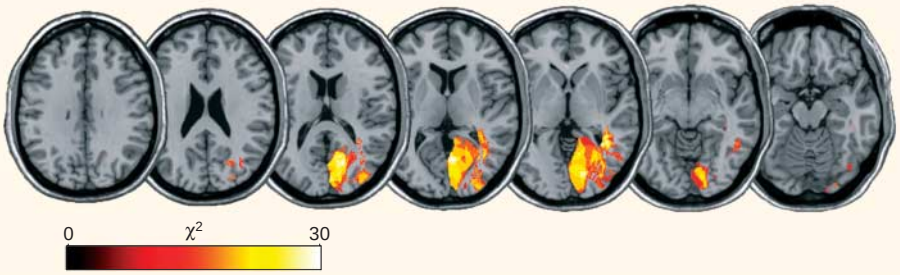

Figure 5 | **Voxel-based analysis of lesions (VAL)** applied to the data described in figure 4. This figure shows the χ^2-distribution which resulted when patients with visual field cuts were compared with patients with intact visual fields. The regions of the occipital cortex and optic radiation shown in orange, yellow and white are statistically significant predictors of visual field cuts (controlled for dependent multiple comparisons using a 1% false discovery rate threshold).

VAL (FIG. 5) is different from VLSM, in that it statistically compares lesion location between different groups, for example a group of patients who show a disorder of interest and a group of control patients with brain damage who do not show the disorder. Furthermore, VAL attempts to address the problem that patients with deficits tend to have larger injuries than control patients who do not have the behavioural problem. For example, patients with large lesions in the left hemisphere are more likely to show speech deficits than those with small lesions. VAL can use logistic regression to ensure that regions are only reported to be statistically significant if they can still predict the deficit even after overall lesion volume has been accounted for. This method reduces our tendency to detect areas that correlate with large lesions but not specifically with the deficit being investigated.

Statistical analysis offers a principled method for lesion analysis. However, these voxel-by-voxel methods raise the same issues of the multiple comparisons problem as neuroimaging studies. Because we are conducting so many tests, we must control for the considerable risk of false positives (claiming a brain region is required for a task when in fact it is not). Conventional methods such as Bonferroni correction will greatly reduce our statistical power (often we will not detect real effects), and a huge number of patients will need to be tested. This is a clear area for future development. We suggest that implementing false discovery rate correction[54,55] might provide reasonable statistical power while guarding against false positives. An application of this analysis is given in FIG. 5. Another direction would be to conduct region of interest analyses — conducting tests for a few anatomically plausible regions, thereby greatly reducing the number of comparisons and pooling data within each region for a more accurate estimate.

Conclusions

We have argued that the lesion method has much to offer neuroscience, despite its limitations. New techniques for imaging the brain and analysing lesion data have the potential to improve the lesion method, and can be used to address several of the common criticisms of this technique. Partly owing to the limitations of other imaging techniques such as fMRI and PET, in our opinion, the lesion method will continue to contribute to our growing theoretical knowledge of the intact human brain. Each technique on its own has only limited explanatory power. However, the strengths and weaknesses of these tools are complementary. Indeed, some brain functions might be

impossible to determine using only the lesion method or functional neuroimaging alone, but can be successfully tackled by combining these techniques[56]. The strength of cognitive neuroscience comes from using convergent tools to investigate the same theoretical question.

For brevity, we have concentrated on comparing and contrasting the lesion method with neuroimaging techniques such as fMRI and PET. Other techniques, such as ERP, MEG and TMS also have important complementary strengths (BOX 1). Finally, we would like to note that, regardless of the method used, the strength of our inferences regarding brain functions always crucially depend on the quality of the behavioural testing. The lesion method, as well as all other techniques mentioned here, can both guide and be guided by the behavioural tasks that are used to investigate healthy individuals[57].

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Competing interests statement

The authors declare no competing financial interests.

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