Neuropsychologia xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neuropsychologia

journal homepage: www.elsevier.com/locate/neuropsychologia

Methods matter: A primer on permanent and reversible interference techniques in animals for investigators of human neuropsychology

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ARTICLE INFO

Keywords:
Animal models
TMS
tDCS
Lesion

ABSTRACT

The study of patients with brain lesions has contributed greatly to our understanding of the biological bases of human cognition, but this approach also has several unavoidable limitations. Research that uses animal models complements and extends human neuropsychology by addressing many of these limitations. In this review, we provide an overview of permanent and reversible animal lesion techniques for researchers of human neuropsychology, with the aim of highlighting how these methods provide a valuable adjunct to behavioural, neuroimaging, physiological, and clinical investigations in humans. Research in animals has provided important lessons about how the limitations of one or more techniques, or differences in their mechanism of action, has impacted upon the understanding of brain organisation and function. These cautionary tales highlight the importance of striving for a thorough understanding of how any intereference technique works (whether in animal or human), and for how to best use animal research to clarify the precise mechanisms underlying temporary lesion methods in humans.

1. Introduction

To attribute a cognitive function to a particular brain region or network, several criteria must be met [see Parker and Newsome (1998) for discussion]. Typically, one might first establish a correlational relationship where brain activity is observed to change in predictable ways during changes in behaviour. To confirm a causal relationship, however, it is critical to *interfere* with the function of that brain region or network and establish that there is a measureable impact on behaviour.

One of the longest-established methods of determining a causal link between a given region or network and a cognitive function is through the study of patients with brain lesions. Classically, researchers infer such causal links when they can show that a lesion to a brain area impairs function A but not function B (a *dissociation*), and especially when they can also show that a lesion to a different brain area impairs function B but not function A [a *double dissociation* Teuber (1955)]. More recently, advances in neuroimaging techniques have improved our ability to map the precise boundaries of lesions, and new analysis techniques such as voxel-based lesion-symptom mapping (Bates et al., 2003) have enhanced our ability to link behavioural deficits with underlying damage (see other papers in this issue). Thus, the fundamental approach of examining lesions in human patients remains one of the most valuable tools for understanding brain function.

And yet, despite the undeniable contributions of patient studies to our understanding of cognition and brain function, they are nonetheless subject to some critical and unavoidable limitations. Conducting studies in animals, while controversial, addresses most of these limitations and thus provides a valuable adjunct to behavioural, neuroimaging, physiological, and clinical investigations in humans.

The purpose of this review is to help bridge the gap between these two approaches. In this review, we will:

- Summarise some of the key limitations of human lesion studies;
- Describe some of the current and emerging techniques for inducing lesions in animals. For the purposes of this review, we primarily focus on those techniques that are currently in common use with non-human primates because they are the animal model of choice for studying higher-order cognitive functions;
- Discuss limitations of animal lesion techniques, including instances where different lesion techniques have yielded different results, thus highlighting the importance of considering methodology when

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http://dx.doi.org/10.1016/j.neuropsychologia.2017.09.019
Received 9 July 2017; Received in revised form 7 September 2017; Accepted 19 September 2017

making conclusions; and
- Briefly comment on potential ways in which animal models can be used to improve our understanding and effective use of reversible techniques in humans.

We do not seek to provide a comprehensive list of all the techniques currently used in non-human species. Rather, we will provide a broad introduction to some of the underlying themes upon which these techniques are based. In doing so, we aim to illustrate how animal models serve to *complement,* not replace, human neuropsychology by addressing many of the limitations for human studies. We will demonstrate how animal models can *extend* human lesion studies by offering new tools, such as genetic approaches, that can tap into the mechanisms that underlie cognitive function in ways that are not possible in humans. Finally, we will highlight important insights from the animal literature when comparing the effects of temporary versus permanent lesions in humans.

2. Key limitations of human lesion studies

For almost 200 years, scientists and clinicians have carefully examined the behavioural deficits of brain-lesioned individuals or groups of patients to infer the function of the damaged brain area. Although the field of phrenology seems laughable today, its founder, Franz Josef Gall, based some of his localisation decisions on examinations of brain damaged individuals (Gall, 1835). Most notably, such patient-based observations led him to ascribe the 'word memory' area to an anterior frontal lobe region that is very near to the area identified by Paul Broca many years later (Broca, 1861; Brown, 1992). Other classic studies include Carl Wernicke's observations on language, John Hughlings Jackson on motor function, and John Harlow on executive function (Critchley and Critchley, 1998; Damasio et al., 1994; Gross, 1999; Harlow, 1848; O'Driscoll and Leach, 1998). Yet, as with any scientific method, human neuropsychology has some limitations for making inferences about cognitive function.

The main limitations are (see also Humphreys and Price, 2001):

- **Location:** Not only is every person's brain unique, researchers have no control over where the lesion occurs or how large an area it covers. Lesions are most typically caused by trauma or stroke. While lesions caused by trauma (e.g., gunshot wounds, blunt force trauma) can theoretically be located anywhere in the brain, lesions caused by stroke are, by definition, dependent upon the underlying vasculature of the brain. This means some brain regions are more likely to be affected than others (Corbetta et al., 2015; Kang et al., 2003; Wessels et al., 2006). For example, strokes will more often involve the middle and not the posterior cerebral artery, meaning that posterior cortex is affected relatively infrequently. Similarly, areas supplied by more than one cerebral artery will also rarely suffer ischemia. From a research perspective, this means that lesions almost never obey cytoarchitectonic borders or functional distinctions that allow researchers to address specific hypotheses about particular areas. It also means that it is highly unlikely that the area of scientific interest will be the only area affected in that patient. Indeed, some of the most influential neuropsychological cases in the scientific literature are thus defined due to the rare location and/or unusual focality of their lesion [e.g., Patient DF, who suffered extensive damage to the lateral occipital complex following carbon monoxide poisoning (Goodale et al., 1994); or Patient TN, who suffered two successive strokes resulting in near-complete bilateral damage to the occipital cortex (de Gelder et al., 2008)].
- **Patient experience:** With patient studies, we have no control over the health and life experience of the participant. Stroke patients are generally older and can have additional co-morbidities. Similarly, altered function in patients who have undergone resections to treat epilepsy could be due to either the surgical lesion, or the neurodegenerative consequences of recurrent seizures and/or associated head injury. This will lead to confounding variables that cannot be completely accounted for in the control group, or difficulty in interpreting brain changes that occur because of the lesion.
- **Time:** Although it is theoretically possible to test patients within the first several days after the initial trauma, these opportunities can be limited by patient drowsiness, the natural and understandable priority for the patient to spend time with visiting friends and family, and/or other injuries sustained by the patient (e.g., if a stroke led to a fall, or there is a brain injury associated with a car accident). So, for both compassionate and logistical reasons, patients are typically seen days, weeks, or even years after the injury; raising concerns about post-lesion reorganisation and/or compensation that might obscure the true function of a given area.

In short, there is an inherent confound in using a *permanently damaged brain* to understand the function of an *intact healthy nervous system.* By contrast, animal models offer the opportunity to study the neural bases of behaviour without many of these limitations. Animal models allow for substantially more control over *where* a lesion is located; potentially with exquisite control over the size/boundaries of the lesion (see below). Animal models also provide control over *when* the lesion takes place (e.g., before or after a given task is learned or knowledge is acquired); and *how soon after* the lesion and *how often* the subject is tested on the relevant cognitive tasks. The ability to test and re-test the subject grants greater statistical reliability and offers the opportunity to further assess recovery of function over an extended timeframe.

Until relatively recently, perhaps the most significant advantage of animal models over human studies was that it was the only way in which it was possible to study the effects of *reversible* lesions. Although reversible lesions do not completely eliminate the possibility for reorganisation, nor do they control for potential off-target effects (e.g., Otchy et al., 2015; see below), they nonetheless provide an opportunity to examine the *immediate* consequences of removing a specific brain region on behaviour. This particular advantage of animal models over human studies might be closing, thanks to the development of 'reversible' or 'virtual' lesion techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) that can temporarily alter brain function in humans (see other papers in this issue). However, these new methods can also create new problems in that the effects can be subtle, the location of stimulation can be uncertain, the mechanisms of action are unclear, and the techniques are currently limited to cortical areas located on the dorsal and lateral surfaces (and not subcortical structures, or cortical structures located on the ventral or medial surfaces).

Perhaps because of these limitations, the findings from temporary inactivation versus permanent lesion studies in humans do not always correspond. For example, Van der Stigchel and his colleagues have shown that oculomotor inhibition is impaired in patients with permanent lesions to the frontal eye fields (Van der Stigchel et al., 2012), but enhanced by temporary inactivation of the same area using TMS (Bosch et al., 2013). Although techniques that temporarily inactivate brain tissue in humans could bypass some of the limitations of permanent lesions, they also raise new questions.

Thus, we have new opportunities – and new challenges. Techniques for inducing reversible lesions in animals have been around for much longer than for humans, and over this time researchers have identified several instances of divergence between results obtained with permanent lesions and those obtained with reversible lesions. These discrepancies were not just due to the presence of reorganisation, but also to methodological differences between different techniques that led to differences in the lesion substructure (such as whether they affected fibres of passage or not). This serves as an important reminder – a cautionary tale – for investigators seeking to use reversible lesion techniques in humans: one cannot necessarily expect the results from